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THE NEUROLOGICAL RELEASE OF RENIN IN MONGREL DOGS

by

© MALCOLM S. McPHEE M.D.

A THESIS

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I dedicate this thesis to my wife Barbara. Without her moral support and many hours of reading, correcting, typing and "ratting" this work would never have been completed.

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read and recommend to the Faculty of Graduate Studies for acceptance, a thesis titled "The Neurological Release of Renin in Mongrel Dogs", submitted by Malcolm McPhee in partial fulfillment of the requirements for the degree of Master of Science (Surgery).

ABSTRACT

Some controversy exists in the literature (34,37,38) regarding the possible role of the sympathetic nervous system in the secretion of renin from the kidney. Carotid occlusion, bilateral vagotomy and manipulation of the renal perfusion pressure were used to study the increased renin output in response to a sympathetic discharge in twenty-nine normal or sodium depleted mongrel dogs.

Control of the renal artery perfusion pressure greatly increased the possibility of "renin" secretion as well as the magnitude of such a release.

Release of "renin" occurred ten to thirty minutes after the onset of the pressor response in spite of the fact that the maximal increase in blood pressure was evident three to ten minutes following carotid occlusion.

The data would suggest that renin release occurred secondary to intrarenal changes which may involve the handling of sodium by the kidney. There was no evidence to implicate a baroreceptor or direct neurological mechanism in renin release following carotid occlusion.

Sodium depletion in mongrel dogs increases the resting levels of renal vein renin. An increased sensitivity for renin release could not be demonstrated statistically in sodium depleted dogs however.

ACKNOWLEDGEMENTS

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HISTORICAL REVIEW

Renal Hypertension:

Choun-You-J, a Chinese physician wrote, in 200 B.C.: "When the pulse upon depressing is very firm, and upon superficial palpation tight, then the disease has its seat in the kidney." (1)

It was not until 1836 that the link between hypertension and the kidney was again recognized. Richard Bright believed that the kidney could cause blood alterations that hindered its flow through small vessels and thus produced hypertension in some pathological processes.

In 1898, Tigerstedt & Bergmann (2) working at the Karolinska Institute, infused rabbit kidney extract to obtain a pressor response. These workers suggested the agent responsible was a protein-like substance which might have clinical significance but they did not propose a physiological mechanism.

Janeway (3) coined the terms "essential hypertension" and "hypertensive vascular disease" in 1908 and was succeeded by several investigators (4,5,6,7) who produced experimental renal hypertension by partial nephrectomy, renal vein stasis, nephrotoxins and renal irradiation.

Fahr (8) suggested in 1925 that renal ischemia might be a factor in the production of renal hypertension but it was not until 1934, when Goldblatt's (9) classical paper appeared, that this concept gained much favour.

Goldblatt demonstrated that renal hypertension could be a sustained mechanism (at least 18 months) if both renal arteries were compromised or one constricted a renal artery ipsilaterally and did a contralateral

nephrectomy. He surmised that his silver clamp had induced renal "damage" by ischemia and that the injury was responsible for the release of some substance capable of producing hypertension. With mild forms of constriction he was unable to demonstrate renal injury in his dogs but felt that more sophisticated techniques would reveal this in future years.

Between 1938 and 1940, Page and Helmer (10) of the U.S.A. and Braun-Menendez (11) in Argentina established the basic reaction of renin with substrate to produce angiotensin. The Americans called the pressor agent "Angiotonin" while the Argentines referred to it as "Hypertensin". In a dual communication in 1958 the compromise name of "Angiotensin" was adopted. Even at this early stage it was recognised that renin itself had no vasoactive qualities but had to react with a plasma pseudoglobulin substrate. They carefully worked out the crude chemical and physiological properties of angiotensin and noted that tachyphylaxis could occur with both renin and angiotensin (although the latter did so only in huge doses).

The histological work of Goormaghtigh (12) in 1939 suggested that the afferent arteriole of the juxtaglomerular apparatus (JGA) was a likely source of renin. These observations have subsequently been supported by many workers using such diversified techniques as fluorescent antibodies, microdissection and elaborate fractionation methods (13,14,15). Very recently Warren (16) found renin in the macula densa as well but this finding has not been substantiated by others.

The amino acid sequence of angiotensin was established by Skeggs (17) in 1956, and Bumpus (18) managed to synthesize angiotensin in 1957. Great impetus was given to work in this field in 1960 when Genest (19) and Laragh (20) established that angiotensin was capable of releasing aldosterone from the adrenal cortex.

Neurogenic Hypertension:

The history of neurogenic hypertension is, interestingly, closely linked with renal hypertension at certain periods but the work is not nearly as extensive.

As early as 1799 Parry (21) demonstrated a link between the carotid arteries and heart rate. Astley Cooper (1836) found that hypertension resulted when one occluded the carotid arteries (22). Cooper attributed this to cerebral ischemia but Siciliano (23) in 1900 felt the pressor effect was too rapid to be caused by such a mechanism. Hering (24,25) and Heyman (26) confirmed this view by defining the carotid sinus reflex between the years 1924 and 1929.

Neurological mechanisms were now clearly involved in some forms of hypertension but several investigators (27,28) believed a hormonal pressor agent was present in the blood of experimental animals with neurogenic hypertension. After Taquini's work (29) this factor was assumed to be adrenalin by most investigators (30) although Hermann (31) did demonstrate a pressor agent was present in blood after adrenalectomy (1939).

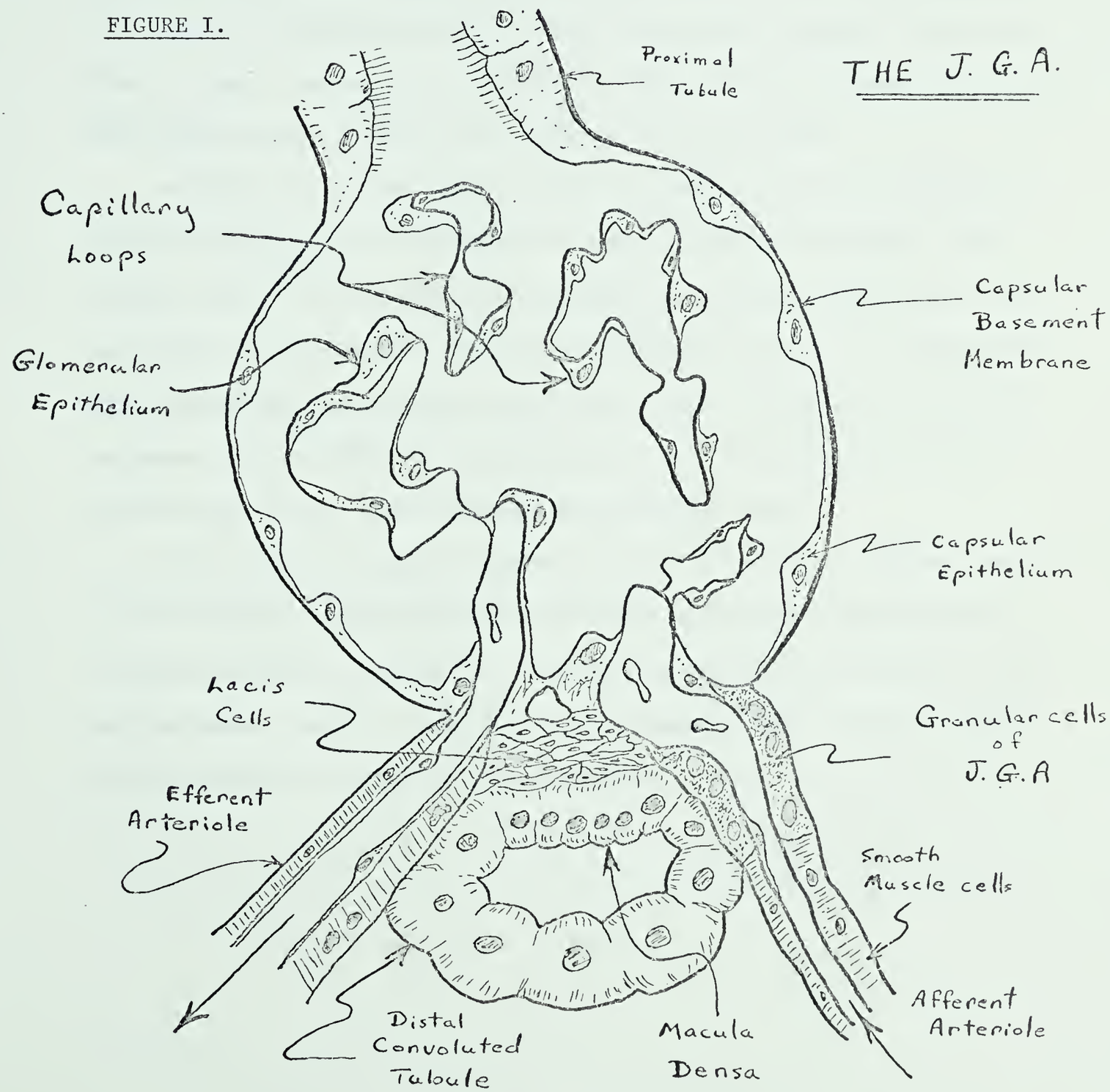
Work lagged relating the release of renin to the central nervous system until the 1960's when reports of elevated renin levels appeared associated with various sympathetic responses (32,33,34,35), catecholamine infusions (36) and following carotid artery occlusion (32, 37,38). Unfortunately, most reports are severely hampered by the lack of a reliable assay for renin or angiotensin and many articles are based on a small series with few or no controls.

ANATOMICAL RELATIONSHIPS

In the past eight years much knowledge has been gleaned from sophisticated microdissection and electron microscope techniques.

Below is a schematic diagram of the relationships of the afferent and efferent arterioles, the macula densa and the lacis cells.

FIGURE I.



It now seems clear that the afferent arteriole has an endocrine function. Many observers (39,40,41) have consistently demonstrated granules in the afferent arteriole with electron microscopic techniques. It is presently believed these granules are related to renin secretion. Barajas and Latta (42) have seen similar granules in the efferent arteriole as well. The constant relationship between the distal convoluted tubule (DCT) and the afferent arterioles has been well documented, but Barajas (39,42) has found in three dimensional studies of the JGA, that a closer anatomical relationship exists with the efferent arteriole! The significance of this report remains to be clarified.

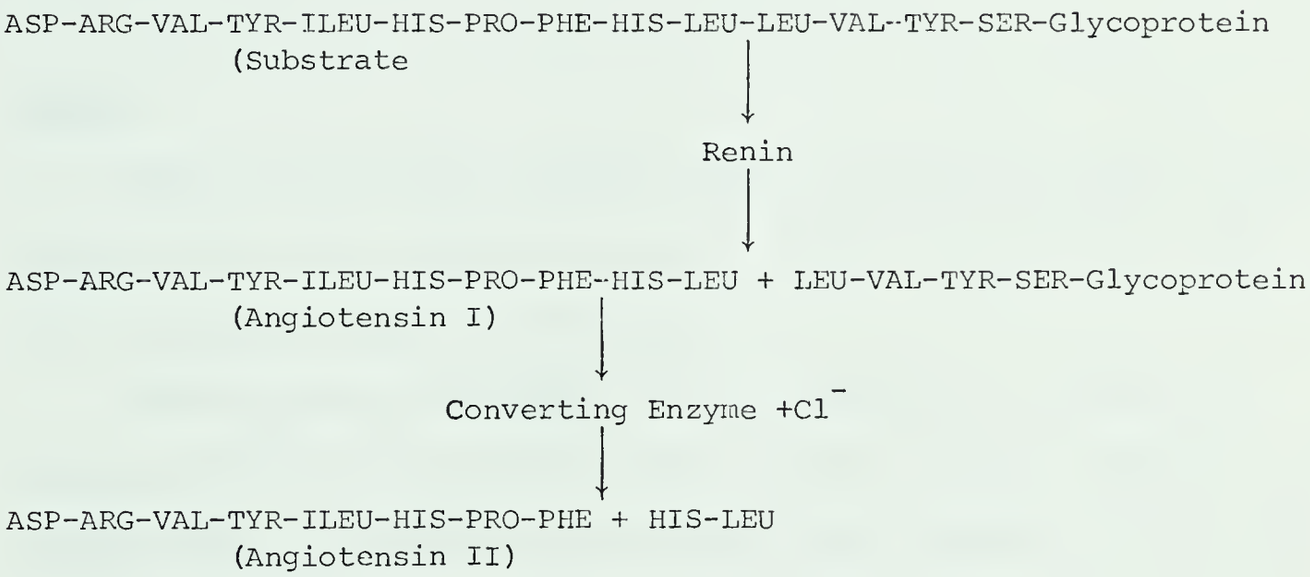
Barajas (43) and Wagermark (44) have demonstrated non-myelinated nerves concentrated in the region of the glomerular arterioles. The latter author used fluorescent techniques to identify sympathetic nerve terminals associated with glomerular arterioles, but saw no relationship with either the lacis cells or the macula densa. This is an important argument against those who would suggest that the sympathetic nervous system might have a direct action on the macula densa.

The intimate relations between the macula densa and granular cells of the afferent arteriole make it tempting to presume a physiological interrelationship, especially as Warren (16) thinks renin exists in the macula densa. However, this has not been established yet and probably will be difficult to prove in the future.

SOME BIOCHEMICAL & PHARMACOLOGICAL ASPECTS OF THE RENIN-ANGIOTENSIN SYSTEM

Basically, present opinion visualizes a release of renin from the kidney which acts on an α_2-globulin blood substrate to form angiotensin I. "Converting enzyme", in the presence of chloride ions, then breaks off the two terminal amino acids from the decapeptide to form angiotensin II. Skeggs (45) has summarized the reaction:

FIGURE II.



Substrate:

Renin substrate has not been obtained in a pure form as yet, but ultracentrifugation methods place the molecular weight at about 57,000 - 58,000 (45). It is currently believed to be manufactured by the liver but evidence for this is tenuous (45,46).

Skeggs (45) found five hog substrate configurations (A, B_1, B_2, C_1, and C_2) using chromatographic techniques. The A, C_1 and C_2 fractions occupied major proportions and all substrate forms had the 14 amino acid sequence outlined above.

Dogs have about 50% of the substrate concentration found in humans (47) and it is believed healthy animals have relatively constant substrate concentrations (48,49,50).

Renin substrate increases in pregnancy, following nephrectomy, and post-ACTH, cortisone and estrogen medications. Hepatectomy, cirrhosis, adrenalectomy and chronic low sodium intake have been shown to decrease substrate levels (47,49).

Renin:

Renin's main function appears to be an enzymatic breakage of the leu-leu bond of the substrate molecule. Deodhar (51) believes renin is a sulfhydryl enzyme in this regard.

Hog renin was fractionated into four analogues by Skeggs (52) although Peart (53) had only one major fraction and two minor ones. The significance of the various forms of renin and substrate is not as yet known.

Peart (53) and Skeggs (52) have recently prepared highly purified forms of renin which seem to place renin's molecular weight ≈40,000. The enzyme is very unstable in pure forms (even in subzero temperatures) although in crude forms it can apparently withstand wide extremes of temperature and pH (46,54).

Angiotensin I and "Converting Enzyme":

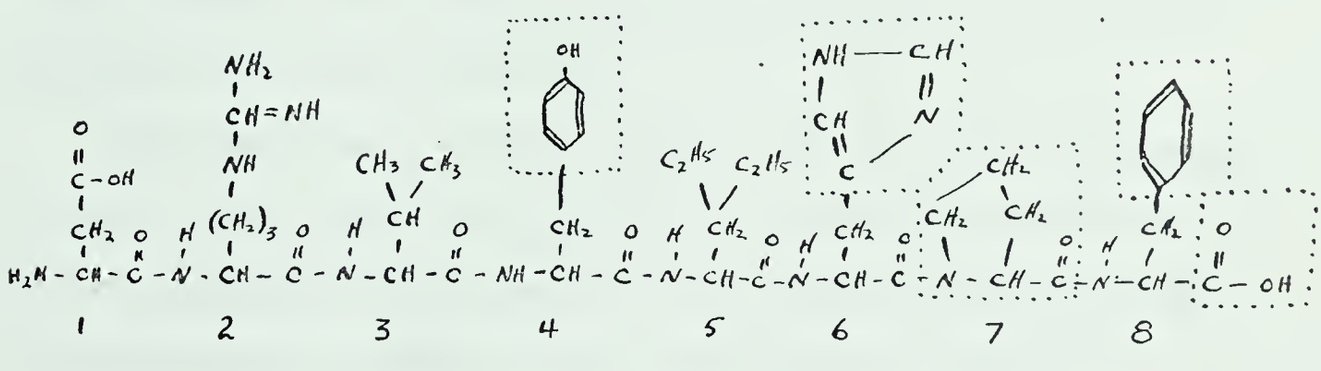
The decapeptide angiotensin I has approximately 20 to 30% of the

vasoactive activity of angiotensin II (17,55). Ng and Vane (55) very recently have presented evidence that angiotensin I is converted to angiotensin II in the lungs. Until this paper appeared it was generally felt that converting enzyme was present only in plasma (17). Bumpus et al (56) in 1960 did suggest however, that homogenates of heart, liver, aorta and ileum contained an enzyme capable of this conversion. Chloride ion has long been known to be a principal activator of converting enzyme (17).

Angiotensin II:

Approximately 80 analogues of angiotensin have been reported to 1966 (57). The basic structure is outlined below.

FIGURE III.



--After Smeby (59)

It seems that an eight carbon chain is essential for activity and the chain may have a helical structure (60). The actual configuration in blood may differ from that occurring when stimulation of the receptor is in progress (58).

Tracer studies (61), utilizing tritiated angiotensin, have demonstrated high concentrations of angiotensin in the kidneys, adrenals and uterus. The agent is the most potent vasopressor known with a physiological reaction that occurs in less then 2½ minutes (62,63). The agent is ten times more potent than adrenalin in normal humans although only two to three times so in the shocked patients (17).

Angiotensin is known to affect various smooth muscles such as intestines, uterus, arteries and veins. The responses are variable according to species and mode of administration of angiotensin (64). Tachyphylaxis develops in dogs and rabbits (65) (in large doses) and therefore prevents a sustained pressor response with continuous intravenous infusion. The effect on precapillary vascular beds is more pronounced than venous beds (66).

Angiotensin has a positive inotropic effect on the isolated perfused dog heart preparation (67) and it is presently believed that no direct chronotropic effect occurs (68). A chronotropic effect may occur secondarily, however, through the release of adrenalin.

Kako et al (69) believe that angiotensin does not result in increased cardiac output although end-diastolic and end-systolic pressures may be increased along with stroke work and the energy used.

Angiotensin stimulates the release of catecholamines from the adrenals but the contribution of this factor in the pressor response has not been elucidated clearly (70).

Evidence that angiotensin affects intrarenal hemodynamics is increasing. Glomerular filtration is reduced in dogs (71) and there is indirect evidence it may affect the renal medullary circulation as well. Recent reports (72,73) suggest that angiotensin may have a natriuretic effect involving a direct action on the renal tubules. This mechanism, however, is far from being understood.

The role of angiotensin in the release of aldosterone has already been mentioned.

Angiotensinases:

At least two angiotensinases occur in plasma which are capable of destroying angiotensin. Many other peptidases, such as pepsin, trypsin, chymotrypsin and carboxypeptidase also do this readily (74).

Angiotensinase A is the principal inactivator of angiotensin II (75,76) and it is said to degrade angiotensin I as well. It does not, apparently, affect naturally occurring substrate. The enzyme is dependent on the presence of calcium ion and therefore is inhibited by EDTA. Its pH optimum is 7.5, a point of importance when considering methods of extraction for either renin or angiotensin.

A second angiotensinase has a pH optimum of 5.5 and is similar in action to chymotrypsin (50). Angiotensinases present in plasma are stable at sub-zero temperatures but lose some activity in about thirty minutes at physiological temperatures.

PHYSIOLOGICAL CONSIDERATIONS

The mechanism of renal "ischemia" and/or hypoxia originally proposed by Fahr (8) and Goldblatt (9) in releasing renin is generally not accepted at present (77,38).

Baroreceptor Theories:

Kohlstaedt (78) in 1940, suggested that a decrease in "pulse pressure" was the primary factor involved in renin release. Some authorities (79) still adhere to this theory today although their numbers are decreasing.

During the last decade Tobian (80,81) proposed a "stretch receptor" function for the afferent arteriole which could respond to changes in pressure and volume. Skinner, McCubbin and Page (38,82,83) have refined this proposal further by saying that the baroreceptor responds to changes in mean perfusion pressure presented to it. A decrease in perfusion pressure of as little as 10 mm Hg is capable of renin release according to these workers.

Vander (84), in a recent review article itemized the stimuli to which such a baroreceptor might respond:

a. Intravascular pressure within the afferent arteriole.

b. Transmural pressure of the afferent arteriole.

c. Tension within the granular cells themselves according to La Place's law relating transmural pressure and the radius of the arteriole.

The stretch receptor mechanism is an interesting theory because the granular cells are ideally situated for such a task and could

release renin with decreased pressure and perhaps inhibit such release if pressures were increased (38).

The concept covers many of the situations which result in renin release but not all of them. It is difficult to visualize a baroreceptor mechanism in situations where renal vein "renin" is elevated without obvious perfusion pressure changes (e.g. sodium depleted dogs).

Macula Densa Theories:

During the past five years interest has arisen in a possible chemoreceptor mechanism to release renin. Thurau (85,86) postulates a physiological relationship between the sodium concentration of the distal convoluted tubule and the afferent arteriole of the glomerulus based on micropuncture studies. Such an association is an attractive hypothesis when one considers the close anatomical linkage of the macula densa and the afferent arteriole.

Brubacher (87) has demonstrated increased "renin" levels in sodium depleted unanesthetized dogs in the resting state. These findings were not associated with changes in the glomerular filtration rate (GFR) or renal plasma flow (RPF). Nash (88) likewise hypothesized a "natriastat" mechanism because he could dissociate renin release from renal hemodynamics but not from some action of sodium. He also suggested that the filtered load and actual sodium excretion were more important parameters to be considered than plasma sodium in the natriastat mechanism.

Dirks et al (89), using micropuncture techniques, have shown that proximal tubular reabsorption is related proportionately to filtered

sodium load. Thus sodium mass entering the loop of Henle, although buffered somewhat in traversing the loop, will be reflected in the distal convoluted tubular sodium load and might be detected by the macula densa. Such experimental findings correlate well with clinical findings and work involving sodium deprivation and saline infusion (90,91,92).

Controversity exists in the actual mode of operation of this mechanism, as Thurau (85,86) believes the exact opposite occurs : i.e. renin release occurs with increased sodium concentration in the D.C.T. Perhaps such controversy will be resolved when the relationship between sodium load, sodium concentration, sodium reabsorption and sodium excretion are established. Giebisch (93) found that hypertonic saline injected into the renal artery actually decreased distal tubular sodium concentration although the mass of sodium excreted was increased. Similar work in the future will probably resolve these present conflicts.

The Role of the Central Nervous System:

Nerve mediated modes of "renin" release have recently been investigated. Such work is propelled by the histological findings of nerve tissue in association with vascular components of the JGA but not at the level of the macula densa (43,44).

Denervation of a kidney reduces the extractable renin by 40% and leads to degranulation of the JGA (94). Vander (36,95) has demonstrated that direct electrical stimulation of the renal nerves results in renin release. Catecholamine infusions into the renal artery will also produce this effect (96). Assuming the erect posture elevates plasma renin levels

(34,97,98) as does hemorrhage (34) and other situations where the sympathetic nervous system is influential. Bunag (32) and Vane (37) have indicated that renin release follows carotid occlusion. Therefore, there is some evidence that the sympathetic nervous system is important in some mechanism which releases renin but how this occurs is not clear.

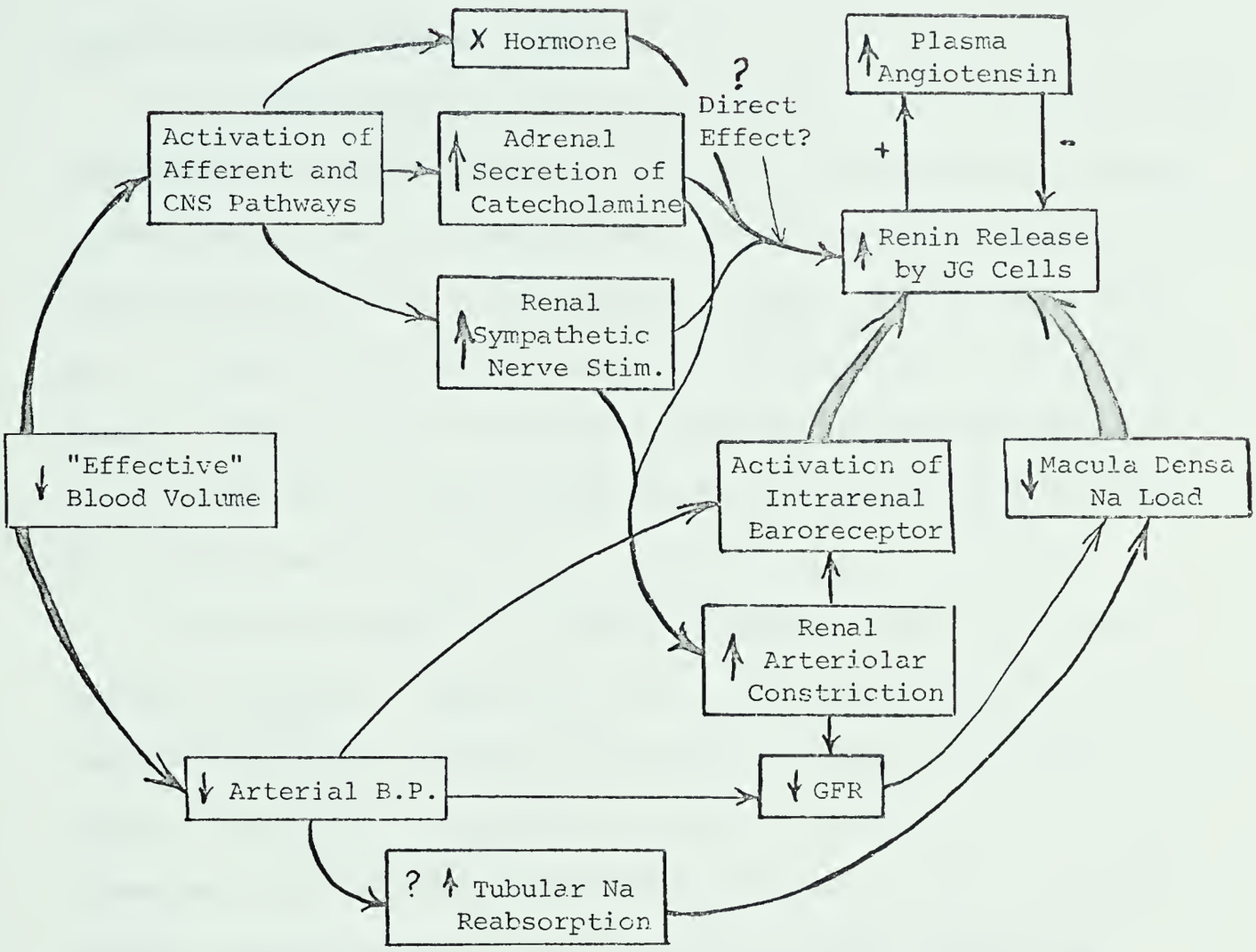
Other States Associated with Renin Output:

Numerous other conditions have been associated with elevated plasma "renin". Ureteral obstruction (92), external renal pressure or inflammation (38,83), pregnancy (99), nephrosis (84), Addison's disease (46,84), cirrhosis (100), Conn's syndrome (84,100) and, occasionally, right-sided heart failure (46,84) are associated with increased "renin" activity.

A comprehensive scheme to correlate many of the mechanisms discussed above has recently been drawn up by Vander (84). (Fig. IV).

FIGURE IV.

Control of Renin Release



— after Vander[84]

THE PHYSIOLOGY OF NEUROGENIC HYPERTENSION

The "vasomotor centre" of the cerebral medulla is a relatively ill-defined region in the reticular formation extending from the obex to the region of the vestibular nuclei and lies between the floor of the fourth ventricle and the pyramids (101).

The carotid sinus is located at the origin of the internal carotid artery and is richly innervated. The nerve of Hering (carotid sinus nerve) passes from the carotid sinus to join the glosso-pharyngeal nerve and thus ascends to the vasomotor centre. Baroreceptors also occur in the aortic arch but use the vagus nerve to reach the vasomotor centre. Chemoreceptors are found in both the aortic arch and carotid arteries and use the vagus and the nerve of Hering to reach the vasomotor centre as well.

The carotid sinus is a "distortion receptor" which can respond to change in pressure. Fibres from this receptor, and the aortic arch, are basically inhibitory fibres to the vasomotor centre. An increase in the number of impulses occurs with an increase in pressure in the carotid sinus and a fall in pressure diminishes the number of tonic impulses. Tuckman (22) has described the function of the baroreceptors as a "buffering system for the outflow from the brain of sympathetic and parasympathetic nervous system activity".

The cerebral cortex, hypoxia and hypercapnia all facilitate vasomotor centre efferent discharge as well.

A decrease in the mean endosinal pressure and pulsatile endosinal pressure (102,103) thus produces a diminution of baroreceptor stimuli

so that the vasomotor centre can respond by:

a. decreasing the distensibility and volume of postarteriolar capacitance vessels.

b. increasing cardiac work

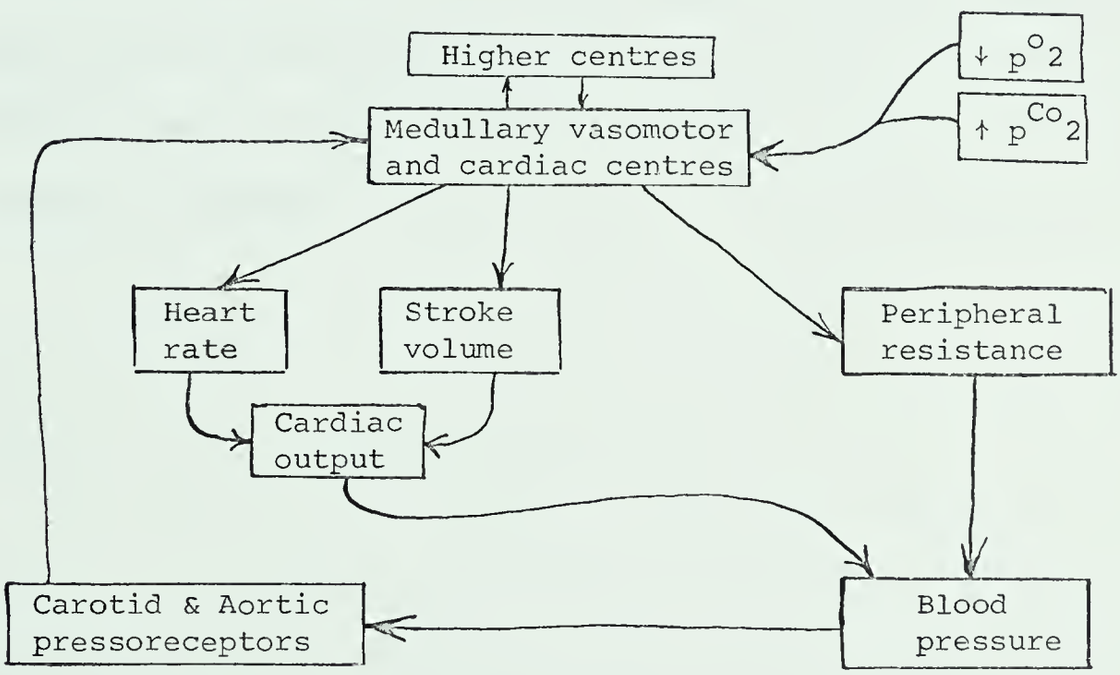
(i) reflexly

(ii) by intrinsic adjustments of the heart secondary to changes in the circulatory system

(iii) by increasing total peripheral resistance

Such a response of course, tends to increase the decreased endosinal pressures.

The mechanisms are seen diagramatically below:



It should be mentioned that these changes occur by both humoral and nervous mechanisms and there is much disagreement in the literature about the exact mechanisms involved in the neurogenic hypertension occurring in response to carotid artery occlusion.

Salisbury (104) believes the response can be explained by an increase in peripheral resistance alone, while others (22,105) have found variable changes in cardiac output and peripheral resistance. Most authorities believe that the cardiac output does not decrease but may increase in some subjects.

The relationship of neurogenic hypertension to renal hypertension is uncertain but there is some evidence such an interaction may exist.

McCubbin (106) has suggested that a renin (renal) mechanism may initiate some forms of hypertension with a "resetting" of the carotid sinus buffering mechanism to maintain the hypertension.

Certainly the work of Kezdi (107) strongly supports such a mechanism. This worker noted that a Goldblatt induced hypertensive state was mild and transient but if associated with denervation of the carotid sinus the hypertension was permanent, stable and "moderate" to "high" in degree.

THE PRESENT PROBLEM.

The literature contains many papers suggesting a relationship between renin secretion and the sympathetic nervous system (32,33,34,35, 36,37,97,98,108). Few papers, however, are based on well controlled, statistically evaluated studies. Many reports are based on a small series with poor assay techniques and often with retrospective evaluation.

The following questions seem pertinent:

1. Does a selective sympathetic response result in renin release from the kidney?

2. If such a release occurs what is the probable mechanism involved?

3. What are the effects of changes in mean renal perfusion pressure on this renin response?

4. Are the sodium stores of the canine subject related to renin secretion secondary to a sympathetic nervous system response?

5. Is renin secretion under such circumstances similar to the well documented release of renin obtained with decreases in mean perfusion pressure and/or pulse pressure?

The experimental procedures were designed so that the intense, selective sympathetic response following common carotid occlusion and vagotomy could be utilized to evaluate possible alterations in renal vein "renin" concentrations. Such a response might be magnified by depletion of the sodium stores of the canine subject and thus provide results which could be evaluated statistically. Careful hemodynamic studies, hopefully, would provide some basis for assessing the mode of release, especially if manipulations of these parameters were studied.

METHODS

Canine Subjects:

Mongrel dogs, weighing 15 to 26 kg., were divided into two major groups. Group A dogs, hereafter referred to as "normal" dogs were fed a standard kennel chow containing 0.5 - 1.5% sodium chloride pre-operatively. Group B dogs, animals designated "sodium depleted", were given 50 mg. of hydrochlorothiazide seven days prior to the day of operation and fed a low sodium diet (Prescription diet(R), Hill Packing Co., Topeka, Kansas) containing less than 1 mEqwt per 100 gms. chow, for the seven day interval.

A 24-hour urine collection was obtained immediately prior to surgery by confining the dog to a metabolic cage overnight and aspirating any remaining urine from the bladder at the time of surgery.

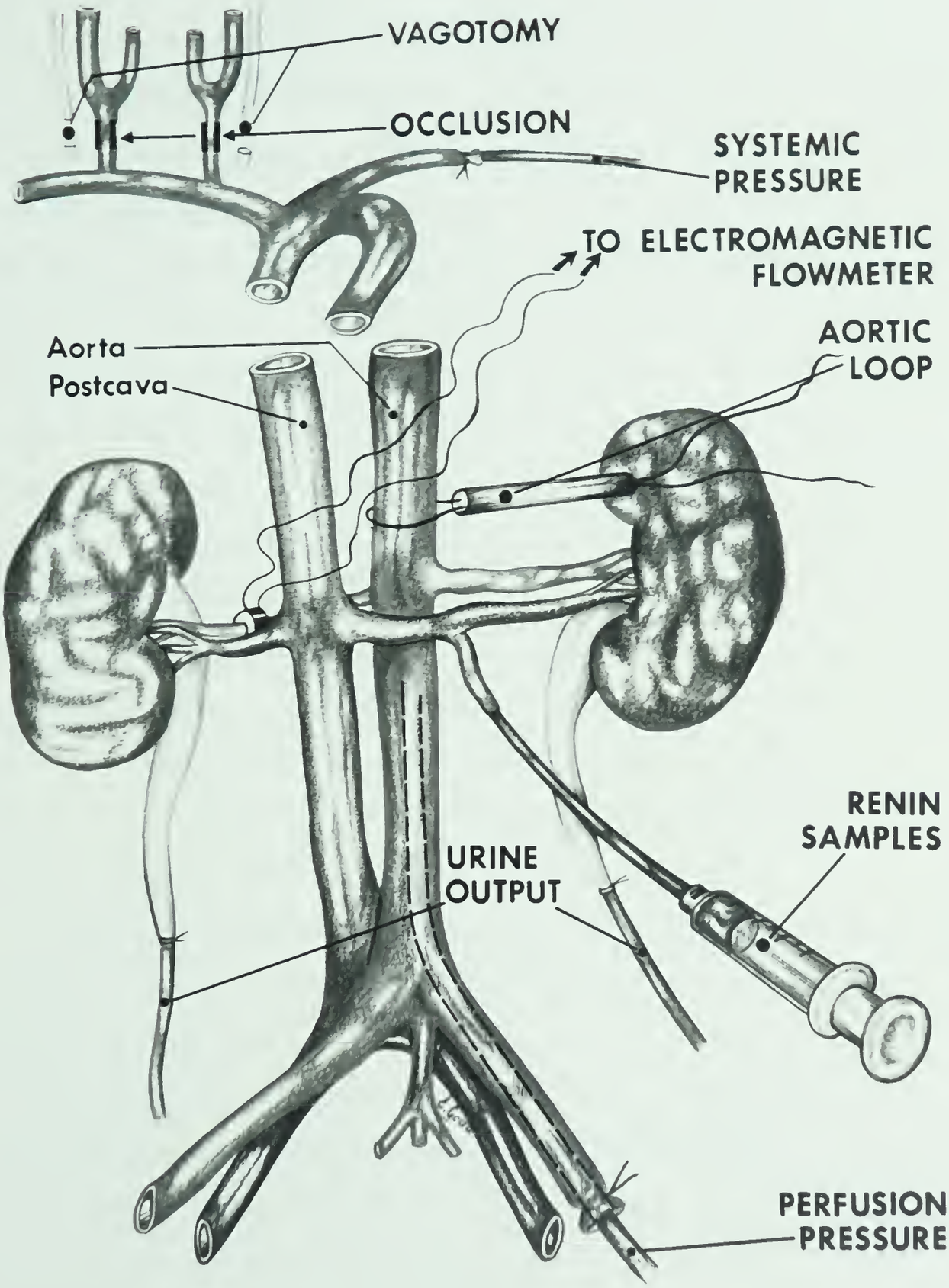
Experimental Surgery: (See Fig. VI)

On the morning of surgery the dogs were anaesthetized with sodium pentobarbital (Diabutal(R) 0.5 cc/kg. BW iv) intubated and placed on positive pressure respiration (air). The carotids were then exposed through bilateral vertical neck incisions. A segment of adjoining vagus nerve was then resected and a length of umbilical tape passed around the common carotid arteries to facilitate exposure of these vessels later.

Polyethylene pressure catheters (P280 - Clay-Adams) were inserted into the left brachial and right femoral arteries. Pressure monitoring was accomplished by means of Statham transducers (P23AA) and a Beckman Dynagraph Recorder.

A midline abdominal incision was used to gain access to the ureters, aorta, right renal artery and left gonadal vein.

FIGURE VI.


VAGOTOMY
OCCLUSION
SYSTEMIC PRESSURE
TO ELECTROMAGNETIC FLOWMETER
AORTIC LOOP
Aorta
Postcava
RENIN SAMPLES
URINE OUTPUT
PERFUSION PRESSURE

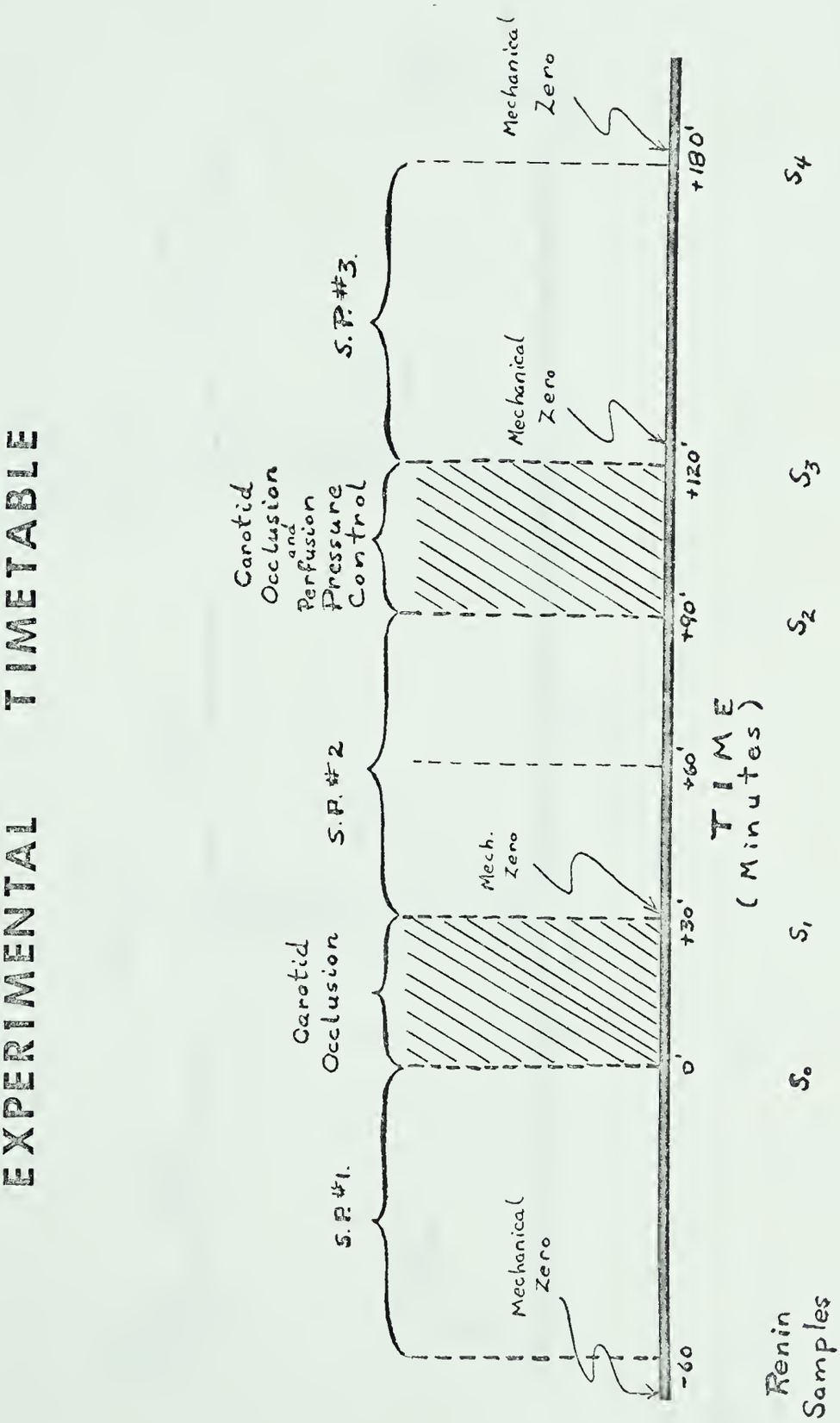
Bilateral polyethylene uretheral catheters (P200) were inserted, secured with a ligature and brought out through stab wounds in the lateral abdomen. A length of umbilical tape was passed around the aorta between the diaphragmatic crura approximately 5 cm. above the right renal artery. A noose was then fashioned by passing the ends of the tape through a glass tube (0.5 cm. in diameter).

The right renal artery was exposed by careful sharp and blunt dissection so that hemorrhage and damage to the renal nerves was minimized. A second noose (see Fig. V) was fashioned around the distal branches of the renal artery and an electromagnetic flow probe (2-3.5 mm. diameter) attached to the main branch of the right renal artery close to its aortic take-off. Great care was used to ensure that a snug fit resulted and that the arterial supply was not compromised. If two renal arteries were encountered, one was used in flow measurements and then only if a good fit could be achieved. Such arteries were usually small but no flow attempts were made using probes less than 2 mm. in diameter. A square-wave, dual channel electromagnetic Flowmeter (Zepeda Instruments - S.W.F.-1) was used in conjunction with the Beckman Dynagraph Recorder for a continuous monitoring of renal flow.

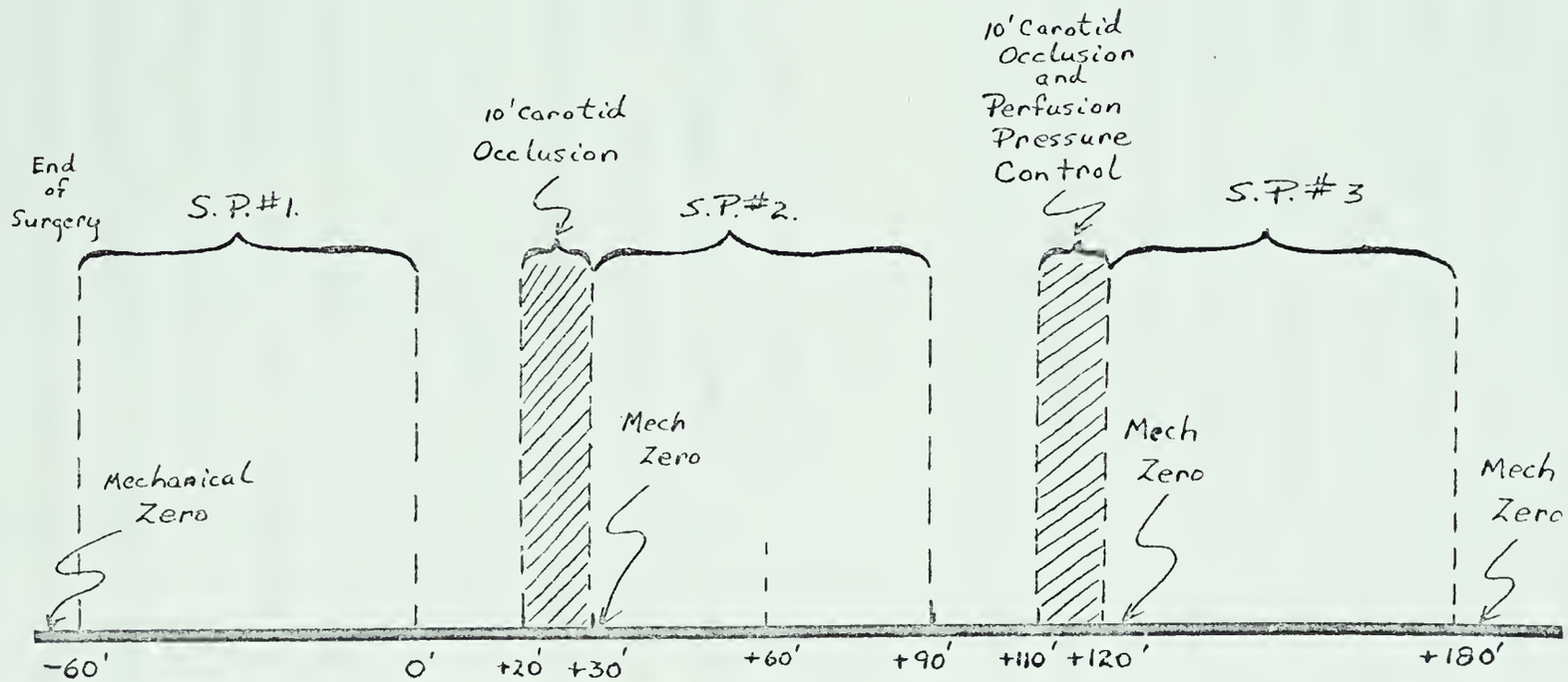
A sampling catheter(Bardic Angiocath$^{(R)}$ #16) was then inserted into the left gonadal vein with its tip lodged 1 to 2 cm. from the renal hilum in the renal vein.

Following the experimental period an autopsy was performed to verify catheter and probe placement, the anatomical conditions of the kidneys and to obtain renal weights.

FIGURE VII.


EXPERIMENTAL TIMETABLE
S.P. #1.
Carotid Occlusion
S.P. #2
Carotid Occlusion and Perfusion Pressure Control
S.P. #3.
Mechanical Zero
Mech. Zero
Mechanical Zero
Mechanical Zero
-60
0'
+30'
+60'
+90'
+120'
+180'
TIME (Minutes)
Renin Samples
S0
S1
S2
S3
S4

EXPERIMENTAL TIMETABLE GROUP IV



*S.P. = Stabilization Period

FIGURE VIII.

Basic Experimental Timetable:

The experimental procedure encompassed a 4-hour period, as depicted in Fig. VII above, following approximately 45 - 60 minutes of surgery. Stabilization periods (S.P.) are seen graphically in Fig. VII.

Bilateral hourly urine collections were made for volume, specific gravity, protein, glucose, sodium and potassium determinations. Blood samples (50 mls)were taken from the left renal vein at the intervals indicated above for three determinations of renal vein "renin" activity. Sodium, potassium, hemoglobin, hemocrit and creatinine estimations were determined from these samples as well.

Mechanical zero (M.Z.) was ascertained at the indicated times by means of the renal artery noose to validate flow measurements. (See Fig.X)

Subgroups:

The two major groups of dogs were then subdivided as below:

1. Group C dogs: This "control" group of dogs underwent the entire surgical procedure outlined but were subjected to the sampling procedures only. Five "sodium depleted" dogs and one "normal" dog comprise this group. The dogs were given 400 - 1500 cc. 5% dextrose in water and pentobarbital i.v. as needed but no other medication or manipulating.
2. Group I dogs: This group is composed of 5 "normal" dogs divided into two subgroups.

   Group Ia dogs had their common carotid arteries occluded with Mixter clamps during the intervals 0 to +30 min., and from +90 to +120 min. of the basic experimental

timetable. During the period +90 to +120 min. the renal perfusion pressure of the renal arteries was kept at, or slightly above, the "control" pressure recorded at the end of the second stabilization period. (4 dogs).

Group Ib dog had the period of renal perfusion pressure control during the 0 - +30 min. interval rather than +90 to +120 min. but otherwise was identical to Ia dogs.

All dogs in Group I were given 600 - 1300 cc. of 5% dextrose in water during the procedure with i.v. pentobarbital in stabilization periods as needed.

3. Group II dogs: This group comprised five "sodium depleted" dogs and was similarly subdivided into two groups so that the period of renal perfusion pressure control could be reversed during the two 30 min. intervals of carotid occlusion.

Group IIa, renal perfusion pressure control achieved during +90 to +120 min. (2 dogs).

Group IIb, control of the renal perfusion pressure established between 0 and +30 min. (3 dogs).

All dogs in this group received 230 - 340 cc 5% dextrose in water intravenously.

4. Group III dogs: Subdivision into two groups was similar to group II but these dogs received 900 - 1400 cc. 5% dextrose in water i.v. during the procedure in an attempt to hydrate them without sodium replacement.

Group IIIa, (four sodium depleted dogs) Renal perfusion pressure control established between o and +30 min.

Group IIIb, (4 sodium depleted dogs) Perfusion pressure control, +90 to +120 min.

5. Group IV dogs: The timetable was altered slightly in this group to see if a correlation between maximal pressor response and "renin" release could be identified. From data obtained in the foregoing groups the maximal pressor response occurred between three and ten minutes after carotid occlusion. The basic design was altered as shown in figure VIII.
The period of carotid occlusion is confined to ten minutes although the intervals between samples are unchanged. Urine sampling was identical to preceding groups. Subgroups were also used so that the period of renal perfusion pressure control could be interchanged.

Group IVa, (3 sodium depleted dogs) Renal perfusion pressure control between +110 and +120 min.

Group IVb, (2 sodium depleted dogs) Renal perfusion pressure control between +20 and +30 min.

All dogs in this group received only 190 - 275 cc. dextrose in water so that there was no possibility of renin inhibition secondary to an osmotic diuresis.

6. Group V dogs: This dog really belongs in Group Ia, but inadvertently received 800 cc. saline i.v. during the procedure.

Electromagnetic Flowmeter Blood Flow Data:

a. Probe Calibration: The S.W.F.-1 flowmeter recorded a flow equivalent in microvolts which then had to be converted to actual flow in ml/min. by means of a standard flow vs. microvolts plot. We found that hematocrit change resulted in errors in absolute flow determinations so that all

probes were calibrated at several hematocrits. The mechanical set-up for determining the calibration graph is seen in Figure IX.

FIGURE IX.

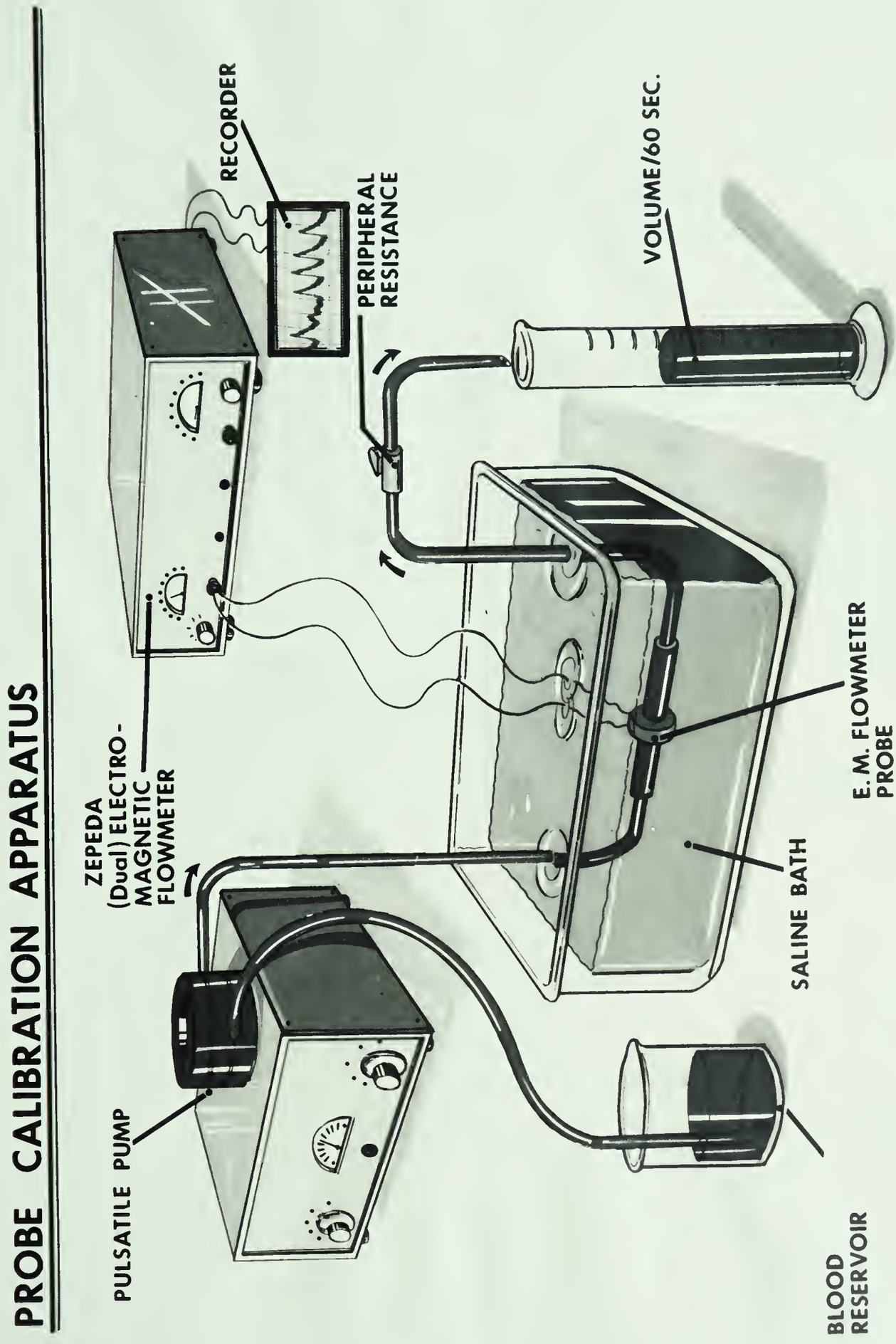
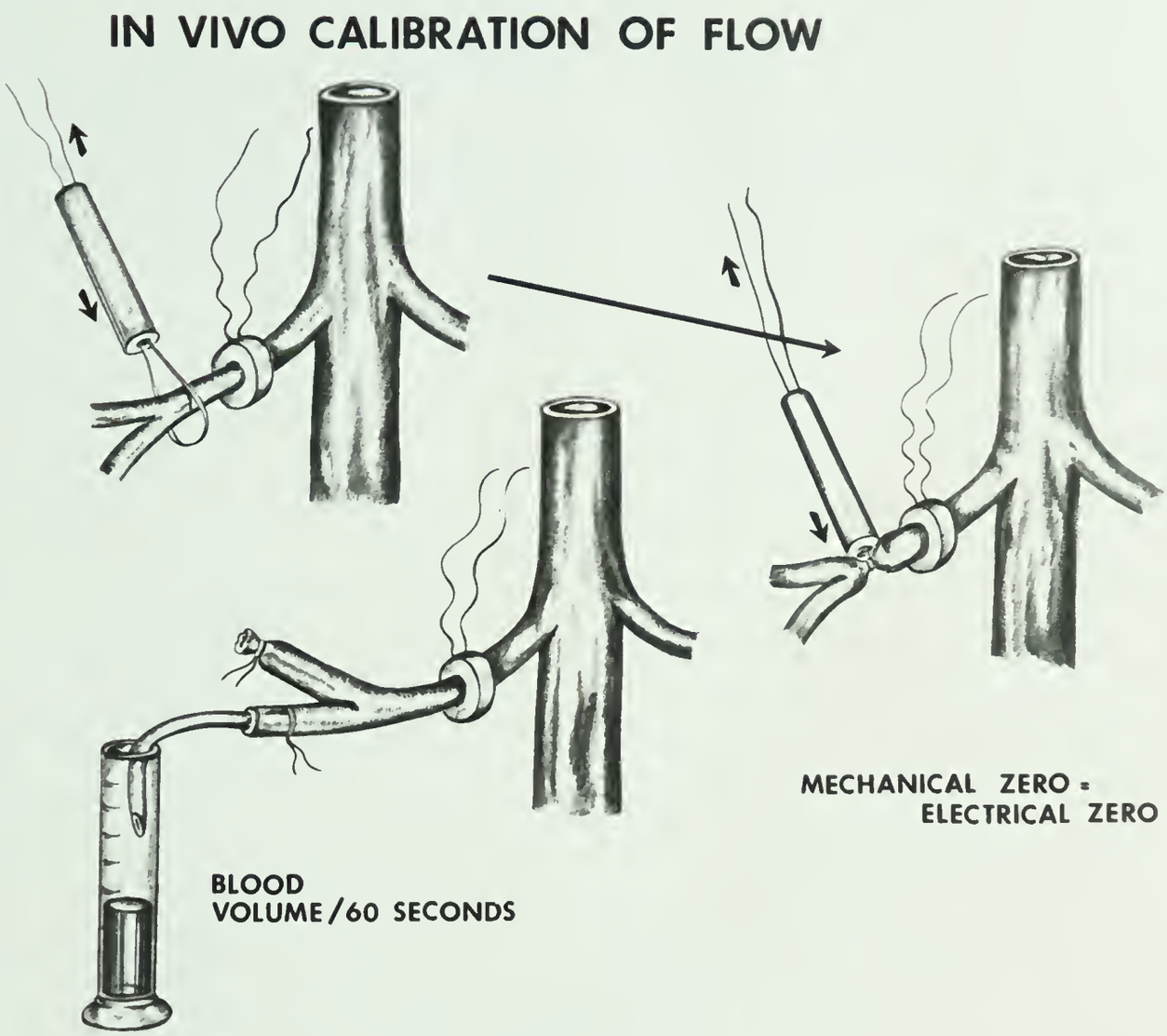
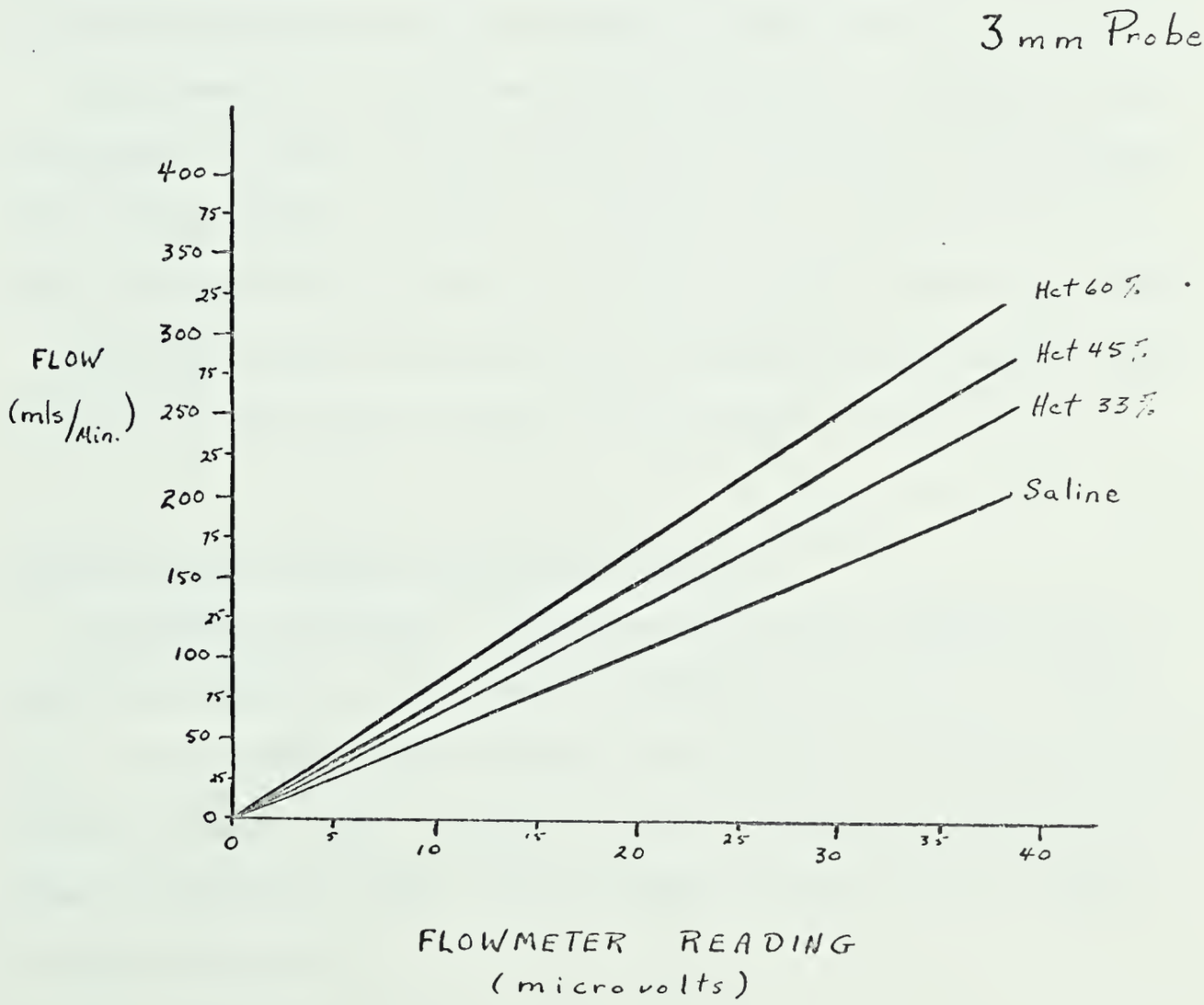

PROBE CALIBRATION APPARATUS
PULSATILE PUMP
ZEPEDA (Dual) ELECTRO-MAGNETIC FLOWMETER
RECORDER
PERIPHERAL RESISTANCE
VOLUME/60 SEC.
E.M. FLOWMETER PROBE
SALINE BATH
BLOOD RESERVOIR

FIGURE X.


IN VIVO CALIBRATION OF FLOW
MECHANICAL ZERO =
ELECTRICAL ZERO
BLOOD
VOLUME/60 SECONDS

b. Corrections for Hematocrit: All determinations were duplicated at three separate sittings using new arteries and new blood. Different hematocrits were obtained by allowing the blood to settle and removing a portion of the plasma fraction. Low hematocrits were obtained by diluting this blood with physiological saline. James (108) reports no difference in correction factors when plasma or saline is used for this dilution. The tracings obtained were similar to that for the 3mm. probe shown below in Figure XI.

FIGURE XI.



All flow data obtained from the dogs was corrected for hematocrit from similar plots obtained for each probe. The data obtained for hemotocrit correction of flowmeter readings were consistent with the work of James (108) but was at variance with data published by Schenk (109).

c. Accuracy of Flowmeter Data: It was found that the probes could detect a 5% change in flow in the range of 70 - 300 ml./min. and that all calibration lines went through zero. This was true however, only when pulsatile flow was used. Gravity-fed, steady flow states resulted in a curving line in the low flow range to reach zero.

An attempt at "in vivo" calibration was made in one dog by direct measurement of flow from the renal artery (see Fig. X). The agreement with calculated flows in this dog was within 4%. This manoeuvre was very difficult however, and did not lend itself to a routine procedure.

Although changes in relative flow could be detected with 5% error, the error in determining absolute flow rate was 10 to 15%.

The Estimation of Renal Vein "Renin Activity":

a. Extraction Procedure: All samples for "renin" estimation were processed according to the method of Warzynski, Demirjan and Hoobler (110). Three samples were analysed simultaneously to determine "renin" activity at the 0 min., +30 min., +90 min., +120 min., and +180 min. times as previously noted. The extraction procedure for each sample is described below:

15 ml. of blood was collected from the renal vein catheter in cold (ice-chilled) heparinized tubes (Riker, Lipohen(R) 1000 usp units/cc L-110). The sample was then immediately centrifuged at 5000 rpm for 10 minutes at 4°C. 6 ml. of plasma was then transferred to a 10 ml. beaker and the pH adjusted to 5.1 using 1 N HCL and a pH meter. 5 ml. of pH-adjusted plasma was then placed in a pre-cooled 25 ml. flask containing 5 ml. of isotonic saline. The sample subsequently was stoppered and stored at -10°C until further processing one to three days later.

The frozen sample was placed in a water bath (37.5°C) with continuous agitation for 31 minutes. Following this the incubated sample was immediately placed in a boiling water bath for five minutes and immediately inserted into a cold-water bath to facilitate the aggregation of precipitated protein.

The sample was centrifuged (International - Model CS) for 10 minutes at 2500 rpm and 6 ml. of the supernatant transferred to a 50 ml. glass-stoppered centrifuge tube containing 3 g. of sodium chloride. The pH was then lowered to less than two by the addition of a few drops of 1 N HCl.

10 ml. of normal butanol was added and the tube agitated vigorously for six minutes. Following five minutes of centrifugation four distinct layers became visible:

(a) Butanol layer

(b) White organic layer

(c) Aqueous layer

(d) NaCl crystal layer

FIGURE XII

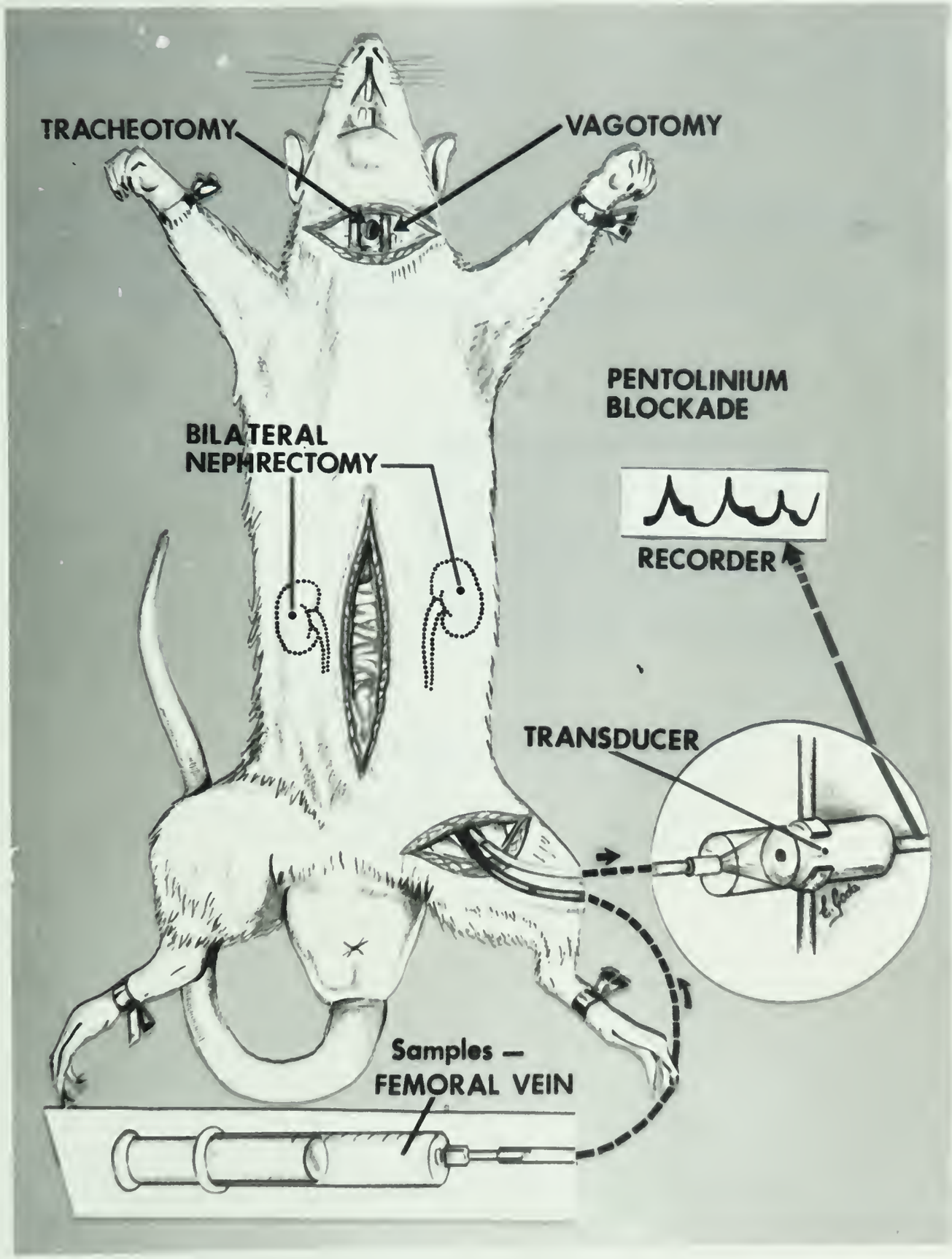
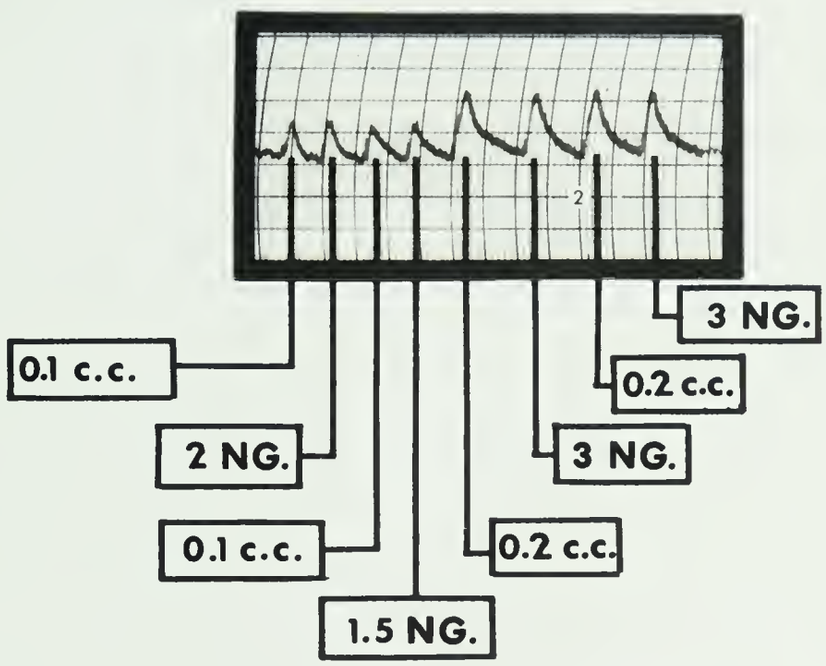

TRACHEOTOMY
VAGOTOMY
PENTOLINIUM
BLOCKADE
BILATERAL
NEPHRECTOMY
RECORDER
TRANSDUCER
Samples —
FEMORAL VEIN

FIGURE XIII.

THE ESTIMATION OF ANGIOTENSIN ACTIVITY



9 ml. of the butanol layer was transferred to a round bottom flask and evaporated to dryness under vacuum in a water bath of 80 - 85°C.

1 ml. of saline was then added to the sample for bioassay to effect a 2.7 fold concentration of the original plasma sample.

Bioassay of Extracted Angiotensin:

Sprague-Dawley and Long Evans Hooded rats, weighing 180 - 300 g. were anaesthetized with sodium pentobarbital (dose 30 mg/kg) intraperitoneally. Bilateral vagotomy, tracheotomy and cannulation (P50 polyethylene catheter) of the left carotid artery were performed through a transverse neck incision. Arterial pressure was recorded on a Beckman Dynagraph recorder via a (Statham) pressure transducer.

A midline transperitoneal abdominal incision was utilized to perform a bilateral nephrectomy and to ensure that the rat was not pregnant. The left femoral vein was cannulated with polyethyline (P50) catheter for sample injection (see Fig. XII).

Ganglionic blockage with pentolinium (Ansolysen(R) Poulenc - 2.5 ng./kg. i.v. and i.m.) was accomplished with subsequent doses given intravenously as needed. Pharmacological blockade increases the response to angiotensin (111, 50) and adds stability to the rat preparation when combined with nephrectomy (112,113). A stabilization period of 1½ to 2 hours preceded assay attempts.

A rat was considered acceptable if its blood pressure response to 5 nanograms (1 ng. = 10^{-9} g.) of angiotensin II (angiotensinamide, Hypertensin(R), Ciba) was greater than 20 mm. Hg and a clear-cut response to 0.5 ng. could be achieved.

Standard Angiotensin II containing 1 ng/.02 ml. was used throughout the study so that no injection (either unknown sample or standard) exceeded 0.25 ml.

Unknown samples were determined at two dosages with direct reference to injected standard angiotensin. Two measurements at each dosage were used routinely (see Fig. XIII).

The sample concentration was then divided by 2.7 to obtain a concentration equivalent to the original plasma sample. Every six samples were accompanied by a control sample containing added standard at concentrations of 0, 5, 10, 15, 20, 25, or 30 ng. per ml. of original plasma. Each of these samples was performed in triplicate and endogenous renin subtracted from calculated values. The control samples were assayed without knowledge of the added standard concentration and served to measure procedural loss and angiotensinase activity.

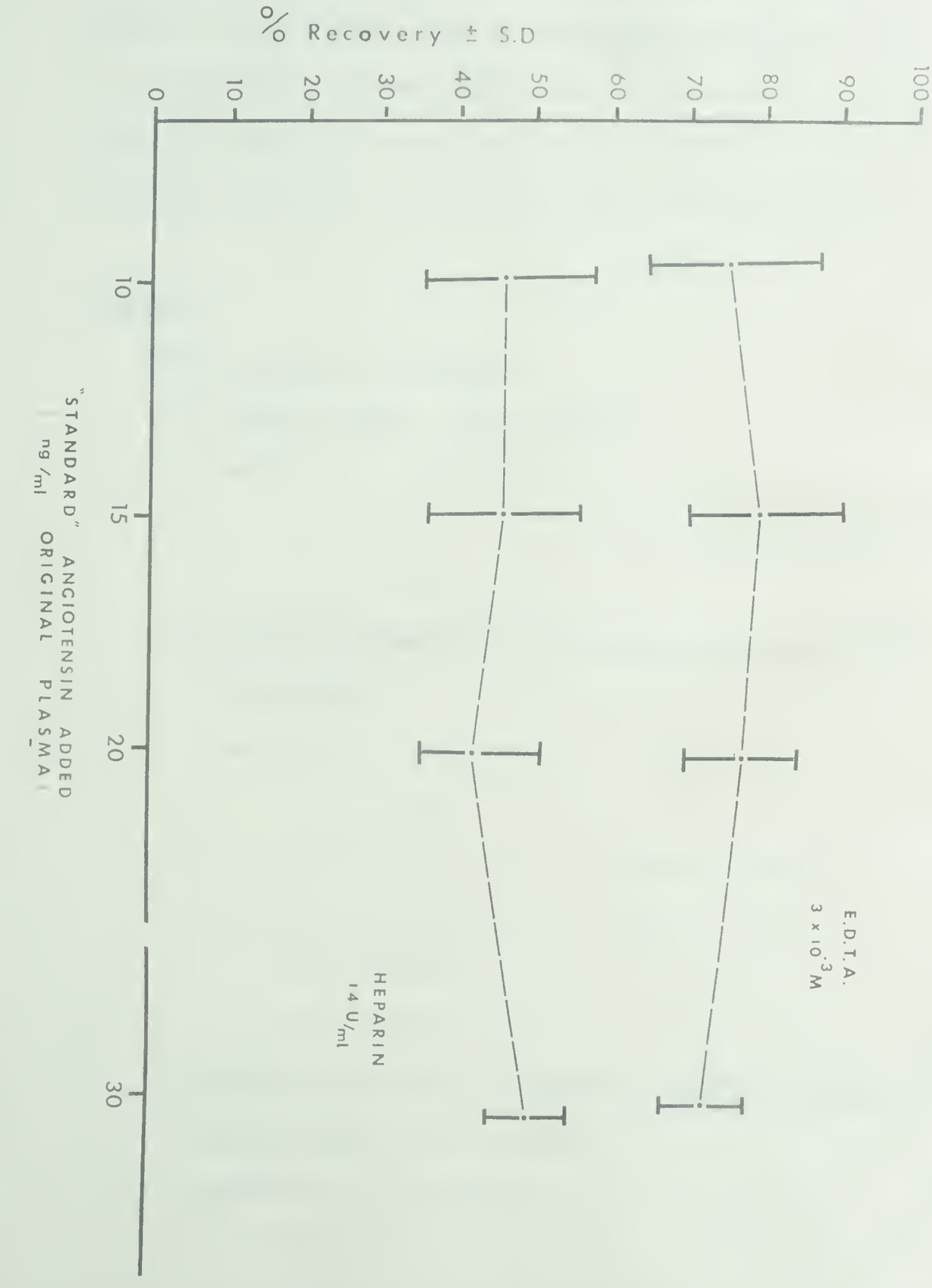
Angiotensinase Activity:

With preliminary attempts at recovering added angiotensin in control samples it became evident that our recovery rate was in the region of 43 - 51%. This was lower than that reported by Hoobler's Lab (75.8 ± 16.9%) and we felt that probably we were not eliminating the effects of angiotensinase destruction.

EDTA is known (Khairallah, 75) to block the action of angiotensinase A by binding Ca^{++} ions required for the enzyme's action.

We subsequently substituted 3×10^{-3} M EDTA for heparin anticoagulation to obtain a 72 - 79% recovery rate. Below is a graph representing the means ± standard deviation of 12 - 17 samples with added angiotensin standard using the two techniques at various concentrations of angiotensin (see Fig. XIV).

FIGURE XIV.


% Recovery ± S.D
0
10
20
30
40
50
60
70
80
90
100
10
15
20
30
"STANDARD" ANGIOTENSIN ADDED
ng/ml ORIGINAL PLASMA
E.D.T.A.
3 x 10⁻³ M
HEPARIN
14 U/ml

Further work established (1) that heparin itself did not inhibit angiotensin (although it may inhibit the reaction from renin to angiotensin II (114) and (2) that the inhibition occurring was present only in the presence of plasma (presumably angiotensinase A). It was further established that EDTA itself was neither pressor nor depressor and was probably eliminated in the aqueous phase of the extraction procedure.

FIGURE XV.

Angiotensinase A. Activity

(a) Control Plasma - Three samples

Sample #1 .37 ng./ml.

#2 .73 " $\bar{x}$ .5 ng./ml. original plasma

#3 .73 "

(b) Saline + Standard Angiotensin II + EDTA (3×10^{-3}M) - six samples

Sample #1 19.2 ng./ml.

#2 17.9 "

#3 19.2 " Recovery rate 91%

#4 16.7 " $\bar{x}$ 18.1 ± 1 ng.

#5 17.9 "

#6 17.9 "

(c) Saline + Standard + 14 units/ml. Heparin (Lipohepin(R) Riker, L-110) - six samples

Sample #1 17.9 ng./ml.

#2 17.9 "

#3 19.2 " Recovery rate 95%

#4 19.2 " $\bar{x}$ 18.6 ± 1.5 ng.

Sample #5 17.9 ng./ml.

#6 15.4 "

(d) 5 cc. plasma + Standard Angiotensin II + 15 units/ml. Heparin - six samples

Sample #1 8.9 ng./ml.

#2 10.7 "

#3 8.9 " Recovery rate 43%

#4 8.9 " $\bar{x}$ 9.1 ± 1 ng.

#5 8.0 "

#6 8.9 "

(e) 5 cc. plasma + Standard Angiotensin II + EDTA (3×10^{-3}M) - six samples.

Sample #1 18.5 ng./ml.

#2 16.7 "

#3 17.4 " Recovery rate 84.5%

#4 18.5 " $\bar{x}$ 17.4 ± 1 ng.

#5 16.7 "

#6 16.7 "

(f) Saline + EDTA (3×10^{-3}M) - three samples

No reaction.

With these findings the method was modified so that all samples were collected in a chilled tube containing 3×10^{-3}M EDTA and taken to the cold centrifuge stage of the extraction procedure. No heparin was used in the animal experiments except in the pressure catheters containing 5% dextrose in water. Each dog received no more than 400 units of heparin during the experimental period via this route.

OBSERVATIONS

Fluids and Electrolytes:

Most of the dogs in the study were somewhat dehydrated clinically although dogs designated "sodium depleted" were much more so. In spite of this observation it was difficult to demonstrate hemoglobin or hematocrit values outside of that range of values considered "normal" in this laboratory. The mean ± S.D. of the Hgb, Hct and plasma Na are calculated below. All values represent the mean of four determinations in each dog and were used to calculate a group mean with its corresponding standard deviation.

FIGURE XVI.

Range	Hemoglobin 15(11-18) g. %	Hematocrit 45(38-53)%	Plasma Sodium 135-160 mEqwt/litre
Group			
* Controls	16.6 ± 1	51 ± 4	143 ± 8
I	15.8 ± 1	47 ± 5	149 ± 6
II	17.0 ± 2	53 ± 5	154 ± 9
III	16.5 ± 1.5	51 ± 4	139 ± 7
IV	16.8 ± 1	51 ± 4	158 ± 7

* *Data exclude "normal" dog.*

Some evidence for dehydration can be seen from this table especially in groups II and IV, but it is certainly difficult to label any dog or group of dogs as dehydrated in a biochemical sense. The unfortunate factor in this data is that we cannot, in the vast majority of dogs,

determine the status of the dogs' sodium stores by the plasma sodium determinations.

The 24-hour urine collections were of value in establishing whether a dog was in fact sodium depleted. Nineteen of the 23 dogs which were subjected to the low sodium diet had total 24-hour excretions less than 5 mEqwts. Twenty-two of 23 dogs had values less than 7 mEqwts. but one dog (E1377) had a sodium excretion of 31 mEqwts./24 hours. (See page 102). Normal dogs excrete 20 - 50 mEqwts/24 hours in health.

Urine collections were made during the procedure to ensure that urine formation was occurring and that renal function was not impaired. One dog was deleted from the study because of an obstructed (surgical) ureter and another aged dog because of excessive urinary protein loss and instability during the procedure.

Three dogs were included in the groups in spite of loss of urinary output from the right kidney. Sudden loss of urine flow was found to be due to catheter obstruction in each case. The ureter was cut above the catheter and allowed to lie in the abdomen unobstructed so that the procedure could continue without interference with the flow probe on the renal artery. At autopsy, the ureters were found to be normal in each case with no signs of hydronephrosis.

Hemodynamic Data:

It was evident that stability of blood pressure and renal blood flow could be achieved over the experimental period utilizing periodic intravenous pentobarbital anaesthesia and positive pressure respiration. All

dogs had a respiratory variation in blood pressure which was accentuated by the ventilation but which never exceeded 25 mm. Hg. during the stabilization periods. All pressure data were calculated by obtaining the mean of three readings over a period of ½ hour in stabilization periods and over 10 minutes during experimental periods. The blood pressure during the pressor period was very unstable in some dogs, but again three readings with calculation of a mean value established a quantitative estimation of the response.

Pulsus alternans occurred spontaneously during the period of sympathetic response in two dogs in group II, three dogs in group III and two dogs in group IV. This usually subsided shortly after release of the carotid arteries but did persist in dog 1706 (Group IV) from +25 min. to the end of the experimental period (+180 min.).

Control Dogs: (Figure XVII)

The five sodium depleted dogs in this group are considered as a group and had a mean systolic pressure of 141 throughout the procedure which varied between 134 and 148. The mean pressure was 107 (104-111) and pulse pressure 49 (49-53). Heart rate means varied between 128 and 132 with an average of 131. Flow data are available on all five dogs and varied between 143 and 149 ml./min. with a mean for the group of 146 ml./min.

The one "normal" dog in this group was stable throughout with the following mean data for the experimental period.

FIGURE XVIII.

Systolic Pressure	139 ± 4 mm Hg.
Mean pressure	101 ± 5 mm Hg.
Pulse pressure	52 ± 3 mm Hg.
Heart rate	89 ± 3 beats/min.
Flow	73 ± 3 ml./min. (2 renal arteries)

Group I: (see Fig. XIX and appendix)

Clamping the carotids caused a profound change in hemodynamics in all dogs. The five normal dogs of this group increased their systolic pressure ($\bar{x}$+84), mean perfusion pressure ($\bar{x}$+68), pulse pressure ($\bar{x}$+16), and heart rate ($\bar{x}$+22). The renal perfusion pressure was elevated 1 mm Hg. above control values and the pulse pressure was decreased by 34 mm Hg. during the phase of modified sympathetic response.

Flow data is available in 3 of 5 dogs which revealed increased flows in two of three dogs (one decreased his flow) during the period of uncontrolled perfusion pressure and carotid occlusion. All three dogs decreased their flow with carotid occlusion and controlled renal perfusion pressure.

Group II: (see Fig. XX and appendix)

This group, as did group IV, reflected the relative dehydrated state imposed by limitation of the fluid therapy. The systolic pressure rose 52 mm Hg. with elevation in mean pressure, pulse pressure and heart rate of 42, 5, and 32 respectively during carotid occlusion. With control of the perfusion pressure the mean pressure was elevated 3 mm Hg.

above control values and the pulse pressure decreased by 22 mm Hg.

Flow data is available for two of five dogs and was decreased in both dogs during the two periods of carotid occlusion.

Group III; (see Fig. XX and appendix)

Carotid occlusion caused a mean increase in systolic pressure of 90 mm Hg. in these eight dogs. Mean pressure rose 64 mm Hg., pulse pressure 27 and heart rate 29. With aortic noose control of the renal perfusion pressure the mean pressure was elevated 2 mm Hg. above control values and the pulse pressure decreased by 34 mm Hg.

Flow data is present for 7 of 8 dogs in this group. Six of 7 dogs increased their flow rates during uncontrolled carotid occlusion (mean increase is 13 ml./min.). With controlled renal perfusion pressure however, all dogs were found to decrease their flow rates ($\bar{x}$ 23 ml./min.). NOTE: Chart 19 considers groups II and III together as a "sodium depleted" experimental group. Data for the groups and individual dogs can be seen in the appendix.

Group IV: (see Fig. XXI)

All dogs in this group reacted in a hemodynamic way, similar to group II. The systolic pressure increase was only 50 mm Hg. with increases of 48 and 4 mm Hg. in mean pressure and pulse pressure. Perfusion pressure control resulted in 5 mm Hg. mean pressure increase over control values and a decrease in pulse pressure of 28 mm Hg.

Heart rate rose a mean 22 beats per minute for the group of five dogs.

Flow data showed a decrease in renal blood flow during both periods of carotid occlusion in two of three dogs. One dog, however, increased his renal blood flow by 3 ml./min. during carotid occlusion with uncontrolled aortic flow and decreased it by 38 ml./min. with control.

The ten minute period of aortic occlusion in this group was sufficient time for all dogs to develop their maximal pressor response.

FIGURE XVII.

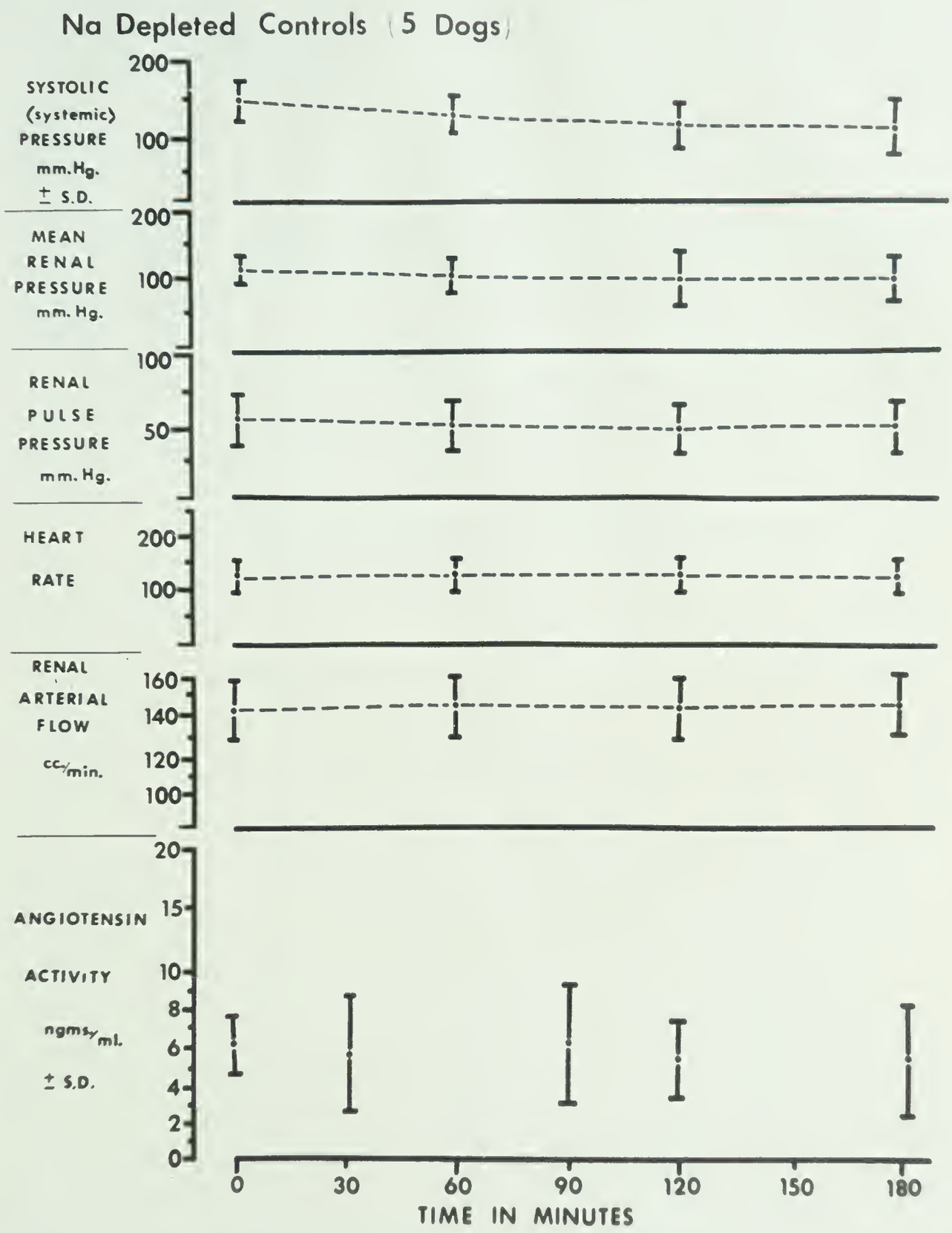

Na Depleted Controls (5 Dogs)
SYSTOLIC (systemic) PRESSURE mm.Hg. ± S.D.
200
100
MEAN RENAL PRESSURE mm. Hg.
200
100
RENAL PULSE PRESSURE mm. Hg.
100
50
HEART RATE
200
100
RENAL ARTERIAL FLOW cc./min.
160
140
120
100
ANGIOTENSIN ACTIVITY ngms./ml. ± S.D.
20
15
10
8
6
4
2
0
0
30
60
90
120
150
180
TIME IN MINUTES

FIGURE XVIII.

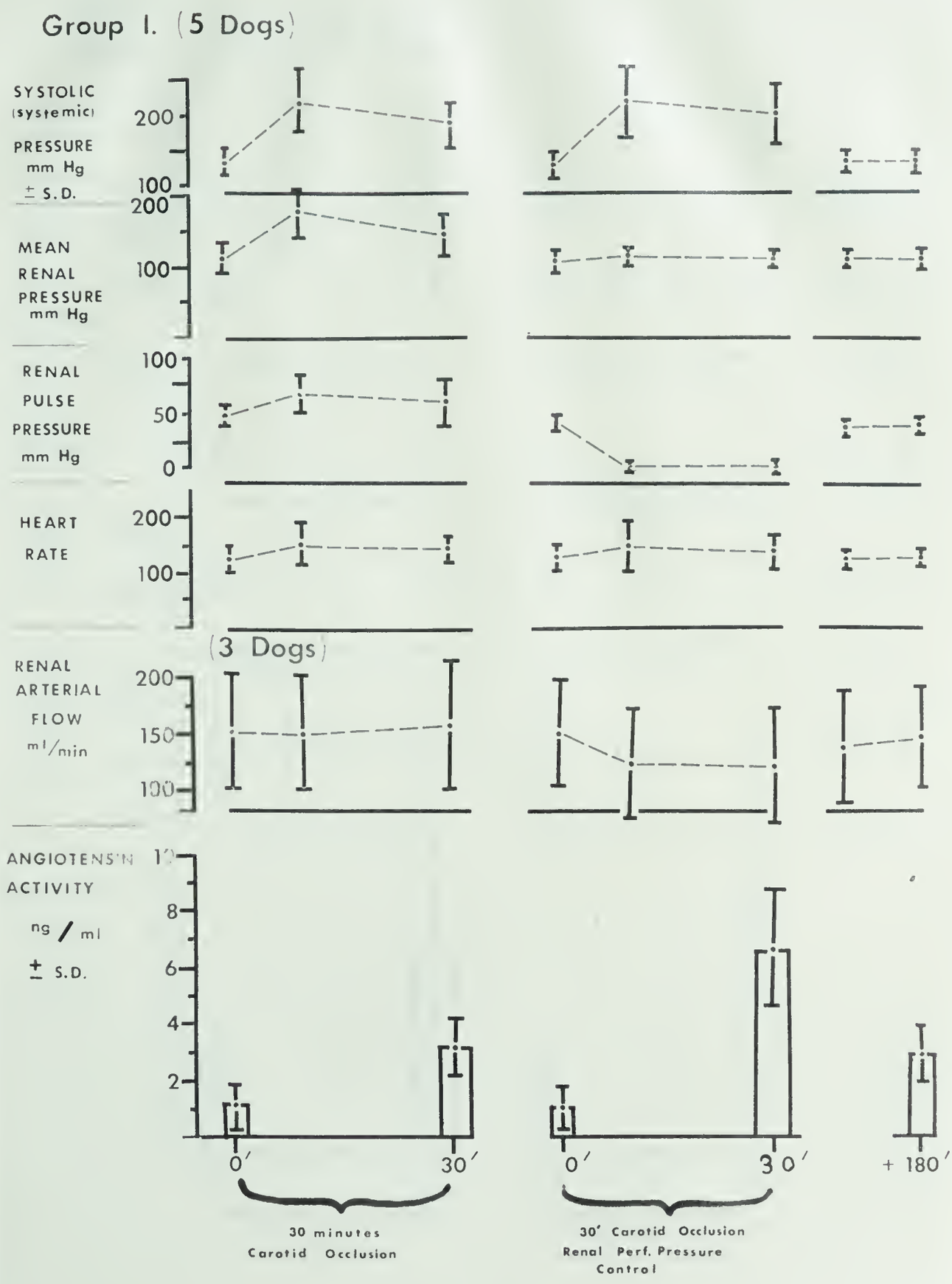


FIGURE XIX.

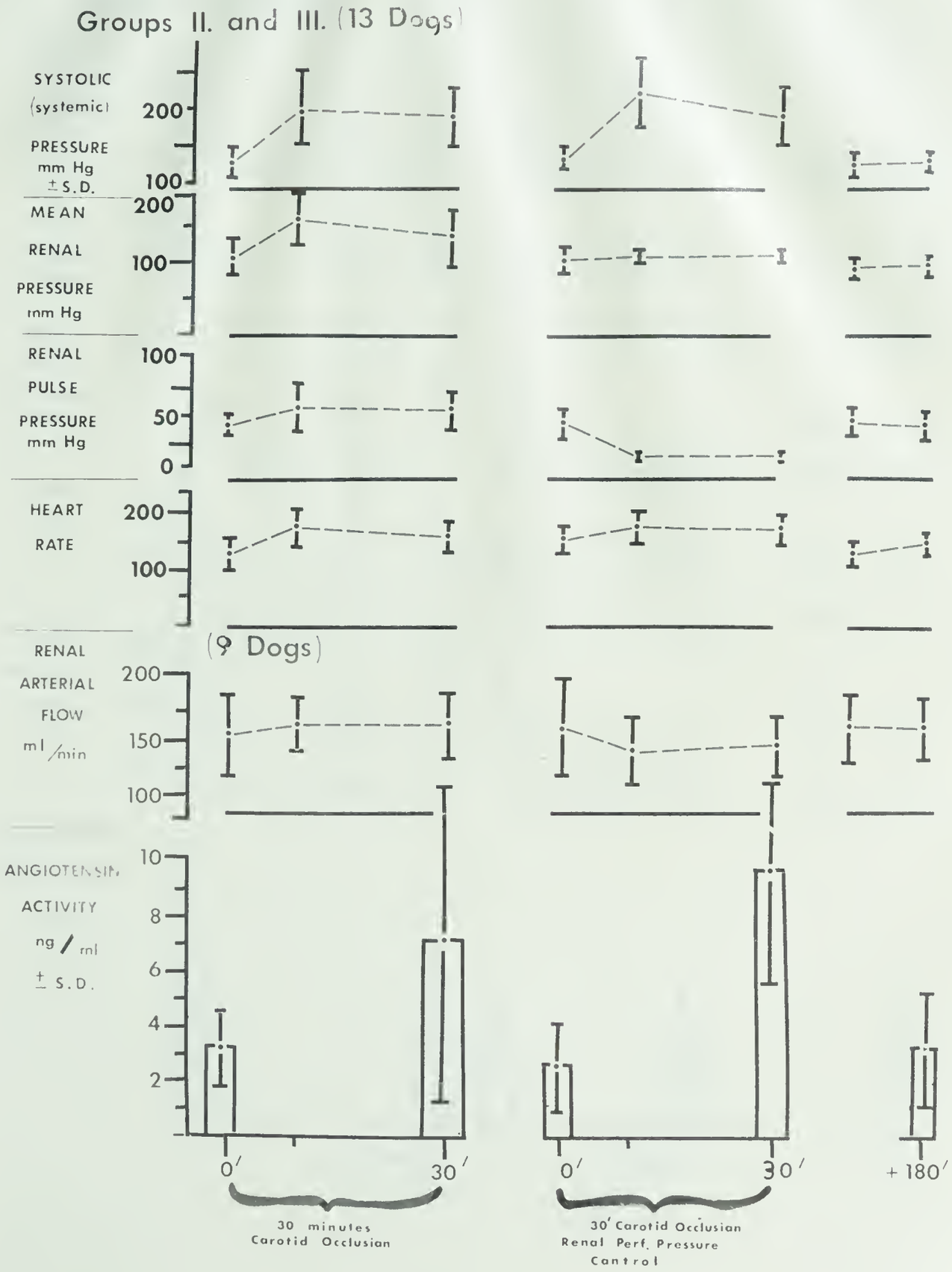
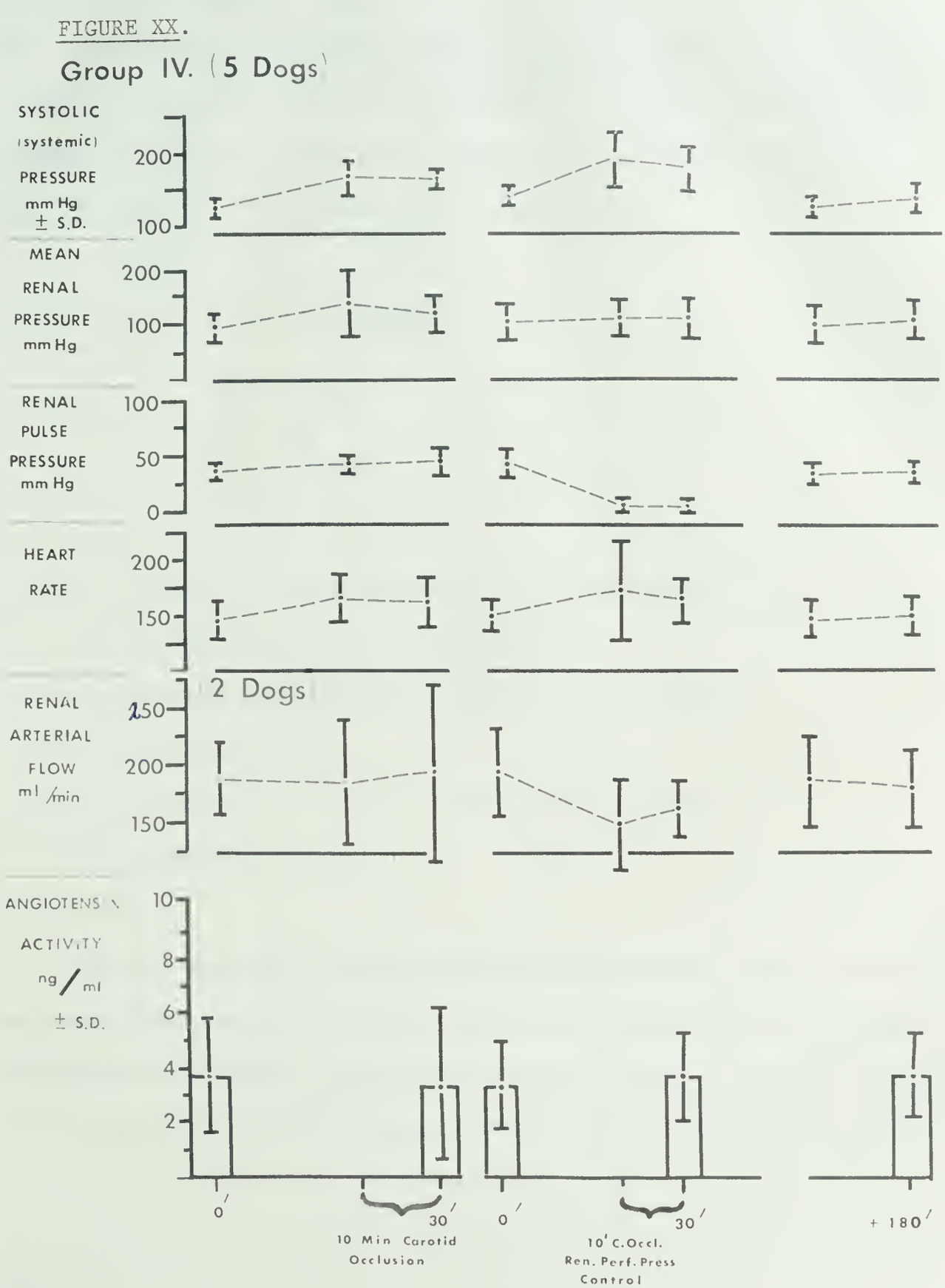

Groups II. and III. (13 Dogs)
SYSTOLIC (systemic) PRESSURE mm Hg ± S.D.
200
100
MEAN RENAL PRESSURE mm Hg
200
100
RENAL PULSE PRESSURE mm Hg
100
50
0
HEART RATE
200
100
(9 Dogs)
RENAL ARTERIAL FLOW ml/min
200
150
100
ANGIOTENSIN ACTIVITY ng/ml ± S.D.
10
8
6
4
2
0′
30′
0′
30′
+ 180′
30 minutes Carotid Occlusion
30′ Carotid Occlusion Renal Perf. Pressure Control

FIGURE XX.


Group IV. (5 Dogs)
SYSTOLIC (systemic) PRESSURE mm Hg ± S.D.
200
100
MEAN RENAL PRESSURE mm Hg
200
100
RENAL PULSE PRESSURE mm Hg
100
50
0
HEART RATE
200
150
RENAL ARTERIAL FLOW ml/min
2 Dogs
250
200
150
ANGIOTENSIN ACTIVITY ng/ml ± S.D.
10
8
6
4
2
0'
30'
10 Min Carotid Occlusion
0'
30'
10' C.Occl. Ren. Perf. Press Control
+ 180'

Renin-Angiotensin "Activity": (See Figs. XIX, XX, XXI).

(a) The Effect of Carotid Occlusion on Renin Release: The Student "t-test" was used to statistically evaluate the mean of differences between control and experimental samples because each dog served as his own control. The method used is seen below:

$$s = \sqrt{\frac{\Sigma (d-\bar{d})^2}{n-1}}$$

$$s_{\bar{d}} = s/\sqrt{n}$$

$$t = \bar{d}/s_{\bar{d}}$$

s : Standard deviation of the mean of the differences

d : Difference between paired samples

$\bar{d}$: Mean of the difference between paired samples

n : Number of dogs

$s_{\bar{d}}$: Standard error of the mean of the difference

t : Student's factor

Each renin sample value used above was the mean of three replicate determinations done on a sample taken at the times shown in the basic experimental timetable. The probability (p) that the increased release of renin was due to chance alone was obtained from standard "t-tables".

FIGURE XXII.

Group	Dogs	Unmodified pressor response "t"	"p"	Controlled renal perfusion pressure "t"	"p"
I	5	6.1	<.01	6.8	<.01
II	5	0.79	>.05	8.1	<.01
III	8	2.59	<.05	4.18	<.01
IV	5	0.63	>.05	0.37	>.05

Controls: Two control periods without carotid occlusion of 30 minutes each were analyzed for each dog.

"t" : 1.43; "p" : >.05

Statistically significant increases in "renin" release occurred if "p" values were less than 0.05 for any one group of dogs.

Control animals did not release additional renin under the experimental conditions outlined.

Groups I, II and III all had increased release of renin if the mean renal perfusion pressure was controlled. Group II, however, did not release significantly more renin during the period of unmodified pressor response, and group III did not achieve as significant an increase as occurred during perfusion pressure control.

Group IV dogs, surprisingly, did not release significantly increased amounts of "renin" during either of the experimental periods.

The single dog (Group V) which was scheduled for group I but inadvertently received 800 ml saline i.v. had no detectable renin activity present in any sample. This dog pointedly demonstrated the antithesis of the scheme to sensitize dogs to release renin by depleting their sodium stores. The failure to demonstrate any renin activity in this dog

emphasized the importance of limiting the administration of sodium during the experimental period. He is mentioned for interest's sake but is not otherwise considered in the thesis data.

(b) The Effect of the Sodium Depleted State:

Sodium depletion in 23 dogs resulted in a mean (control) resting "renin" level of 3.9 ± 2.0 ng./ml. original plasma. The corresponding values for six normal dogs was 1.4 ± 0.8 ng./ml. "Student" statistical evaluation produced a "t" value of 5.18 and a "p" equivalent of <0.001 for this difference.

An attempt was made to assess the magnitude of increased "renin" release occurring in normal and sodium depleted animals. The results of a comparison between differences in group I and groups II and III are shown below:

(i)	Unmodified pressor response	Mean of difference	Standard deviation
	Group I	1.9	± 0.7 ng./ml.
	Groups II and III	3.8	± 5.4 ng./ml.
	"t" : 1.23; "p" : >.05		
(ii)	Control of renal perfusion pressure	Mean of difference	Standard deviation
	Group I	5.4	± 1.66 ng./ml.
	Groups II and III	7.0	± 1.3 ng./ml.
	"t" : 1.23; "p" : >.05		

From this data the apparent difference in means between normal and sodium depleted dogs could not be verified statistically.

DISCUSSION

(a) Renin Estimation:

Although our modification of Hoobler's method for estimation of "renin" activity improved the recovery rate of angiotensin by 25%, any method using a bioassay technique cannot be considered ideal. Even the elaborate methods of Boucher (115) for plasma angiotensin and renin activity utilize the rat assay in the final estimation. In the midst of the work described in this thesis, radioimmunoassays for the determination of angiotensin and renin activity were reported (116,117). Page (116) claims virtually a 100% recovery rate with his immunoassay. Techniques like this will help resolve many of the conflicting reports using older bioassay methods.

In our hands, Hoobler's method was reproducible for renin activity estimation if EDTA was used as an anticoagulant and inhibitor of angiotensinase A. Unfortunately, we do not have a source of renin to determine the actual recovery rate of "renin". If variations in substrate occurred in our dogs our recovery rates would be inaccurate (118). It is worth noting however, that any error in our methodology would result in low renin concentrations so that no false positive results can be expected in those dogs releasing renin. We may, of course, be overlooking some dogs responding in a similar manner, but who are unable to produce a biologically significant increase in angiotensin concentration because of insufficient substrate.

Sealey (114) has reported an inhibitory action of heparin on angiotensin formation from substrate at concentrations of 5 - 1900 units/cc. It was not possible to measure this portion of the reaction.

It is conceivable that such an inhibition was occurring. We have demonstrated that heparin itself had no direct inhibitory effect on angiotensin in the absence of plasma factor. As this inhibitory substance was neutralized by EDTA we presume we have corrected the effect of angiotensinase A activity present in the Hoobler assay.

(b) Sodium Depletion and the Release of Renin:

The method utilized in this study to achieve depletion of sodium stores in the experimental animals was reproducible. Renal vein renin activity measured after one hour of stabilization was statistically greater in sodium depleted dogs when compared with similar samples taken from "normal" dogs. The magnitude of the renin response to carotid occlusion was apparently greater in sodium depleted animals, but this could not be verified statistically. These data agree with the work of Brubacher (87) who reported increased resting levels of renin in the sodium depleted unanaesthetized dog. Bunag (32,90) has also demonstrated a more consistent secretion of renin following hemorrhage, aortic constriction and following the infusion of norepinephrine in the sodium depleted canine subject.

(c) The Hemodynamic Effect of Carotid Occlusion:

The hemodynamic changes which occurred secondary to carotid occlusion were predictable. Carotid occlusion, in these experiments, caused a maximal elevation of systolic, diastolic and mean blood pressure three to ten minutes after the Mixter clamps had been applied. In every instance the pulse pressure and mean blood pressure were increased. The magnitude of the pressor response reflected the state

of hydration. Dogs which were relatively well hydrated (i.e. Groups I and III) had greater increases in blood pressure following carotid occlusion than dogs which had their fluid administration limited (i.e. Groups II and IV).

During the period in which the renal perfusion pressure was controlled similar pressure changes were evident systemically. These were documented by a pressure catheter in the left brachial artery. Predictable hemodynamic changes occurred when the periaortic noose controlled the renal perfusion pressure. In all dogs control of the renal perfusion pressure resulted in a decrease in pulse pressure to the kidneys. The mean renal perfusion pressure was easily controlled at or above the control pressure during this interval.

(d) The Mechanism of Renin Release.

Present authorities (36, 37, 38, 39, 78) believe that most stimuli capable of releasing renin from the kidneys operate either by stimulation of a baroreceptor mechanism or a detector of sodium load in the kidney. Controversy occurs when one tries to determine which of these mechanisms is most important or whether in fact two mechanisms exist. The neurogenic hypertension induced in our dogs invariably produced increases in mean perfusion pressure and pulse pressure. It was therefore possible, due to these opposing stimuli, to separate a baroreceptor mechanism from some other mechanism of renin release.

It is presently believed that a baroreceptor located in the afferent arteriole of the glomerulus responds to a decrease in mean renal perfusion pressure by secreting renin. An increase in renal perfusion

pressure results in an inhibition of renin secretion by this receptor (38).

If a baroreceptor mechanism is the most important method of renal secretion of renin, then the dogs should have had decreased levels of renal vein "renin" during the phase of unmodified sympathetic response following carotid occlusion. In our study groups I and III released renin under these conditions. The magnitude of the renin release was not as great as that occurring during the period of control of the renal perfusion pressure however. These results would suggest that an increase in mean renal perfusion pressure (or pulse pressure) may inhibit the release of renin but it is not capable of obliterating the renin response.

Renin "secretion" following sympathetic response with control of the renal perfusion pressure was more predictable. Groups I, II and III "secreted" renin in this interval. During this period of sympathetic response the renal perfusion pressure was at or slight above control levels and consequently could not have contributed to the release of renin via a baroreceptor mechanism operating in the manner described by McCubbin and Page (38). The pulse pressure during this phase was drastically decreased, and could conceivably have contributed to the renin release if the baroreceptor is sensitive to changes in pulse pressure. Kohlstedt (78) and Indeglia (79) would favour such an interpretation. Udeh (119) has presented rather convincing evidence that pulse pressure change is not an important feature of renin secretion.

This work has been supported by Page (38,32,83).

Renal blood flow data from the experimental dogs indicated that during an unmodified sympathetic response the renal blood flow tended to follow the increase in renal perfusion pressure or remain at control flow rates. During the phase of sympathetic response with control of the renal perfusion pressure most dogs had decreases in their renal blood flows. It is evident, however, that a decrease in renal blood flow is not mandatory for the release of renin. Although increases in "renin" output were more likely to occur if the renal blood flow decreased in our dogs, this parameter is probably not the prime stimulus for renin release.

There is some experimental evidence (120,121,122) that intrarenal redistribution of blood flow can favour an increase in renal medullary flow and that changes in renal blood flow may not necessarily reflect change in glomerular filtration rate. Carriere (120) has suggested that the pattern of renal cortical flow following a sympathetic response induced by hemorrhage is similar to that produced by electrical stimulation of the renal nerves. Castenfors (122) recently found that the subjects under the stress of exercise decreased their renal blood flow more than their glomerular filtration rate. It would, therefore, seem that changes in renal blood flow do not necessarily reflect changes in glomerular filtration rate. With these studies in mind, dogs showing some increase in renal blood flow yet releasing renin (as is evident in the phase of unmodified sympathetic response) may have intrarenal changes which are not directly reflected by renal flow measurements.

Three papers have been published attempting to document the release of renin following occlusion of the carotid arteries (32, 37, 38). Skinner et al (38) found in a very small series of dogs, that carotid occlusion inhibited the release of renin occurring during a decrease in the mean renal perfusion pressure. They were unable to demonstrate renin secretion in dogs which had their carotid arteries clamped in association with vagotomy. Hodge, Lowe and Vane (37), using a technique which detected 10 to 20% of infused angiotensin standard, were able to demonstrate renin release following carotid occlusion in 10 of 17 dogs. Bunag et al (32) in 1966, controlled the renal perfusion pressure during carotid occlusion in seven dogs and obtained renin release in all seven. Unfortunately, all three papers lack control animals and statistical evaluation. Dextrose in saline was used in the studies of Skinner (38) and Hodge (37) and may have obscured their findings.

The data obtained in our study contributes statistical evidence that the sympathetic nervous system can cause the release of "renin" from the kidney (37,117). It is unlikely that a baroreceptor mechanism is responsible for such release. A receptor of increased mean pressure of pulse pressure however, may modify the neurologically induced release of "renin". It is clear that the renin response to a sympathetic nervous stimulus requires more than 10 minutes to increase renal vein plasma "renin" concentrations. Such a lag phase would suggest that (1) the stimulus is a weak one and/or (2) the renin release in these dogs occurs by a different mechanism than that used when renal perfusion pressure is decreased.

A direct neurological release is plausible, but the nerves are associated with both afferent and efferent glomerular vessels (43,44), and are unmyelinated nerves like those associated with peripheral vessels in general. Our data would suggest, by the indirect evidence of flow changes, that an increase in renal vascular resistance is occurring during the sympathetic response. Similar findings have been found by others (34,36). Vander (36) has found that such an increase in resistance is associated with a decrease in glomerular filtration rate and sodium excretion. With evidence of such intrarenal changes it seems more likely that a mechanism involving the renal handling of sodium is operating as proposed by Nash (88). It would be difficult at the present time to prove that our dogs were not releasing renin by a change in sodium load to the distal convoluted tubule. The time interval required for renin release is compatible with such a mechanism. There is indirect evidence (renal blood flow data) that a decrease in glomerular filtration rate occurred in may of our dogs as well.

We have no evidence to base the findings on a mechanism involving specialized nerves to the granular cells of the afferent arteriole or the macula densa. It is conceivable that such a mechanism might exist but one would expect secretion to be identical whether or not renal perfusion pressure was controlled.

Our data would suggest that at least two mechanisms of renin release are operative. The first, a baroreceptor mechanism, releases renin in response to a change in mean renal perfusion pressure in less than 60 seconds (38) and may be capable of inhibiting renin release by other

mechanisms. The second mechanism requires at least ten minutes to release renin and may respond to the load of sodium presented to the distal convoluted tubule. This mechanism could operate in an indirect fashion secondary to changes in glomerular filtration rate and would be operative if vasoconstriction of the afferent arteriole occurred.

Other mechanisms are, of course, possible. A specialized sympathetic nerve may directly cause renin release but if it does the release can be partially inhibited by an elevation in mean renal perfusion pressure and/or pulse pressure. A pulse pressure mechanism is difficult to visualize as the stimulus for renin release in our dogs.

The clinical significance of the data obtained in this study remains uncertain. Control of the mean renal perfusion pressure in dogs either accentuates the renin release following a sympathetic response or allows it to be manifest by inhibiting the influence of an increase in mean perfusion pressure. It is possible that lesions of the renal artery in man may act in a similar manner to the periaortic noose in our dogs. A lesion of the renal artery which limited the increase in mean renal perfusion pressure occurring during a physiological sympathetic response would more likely result in an elevation of renal plasma renin concentration than one which allowed the renal perfusion pressure to act at the level of the afferent arteriole. It may be that sympathetic responses as mild as that occurring with assumption of the erect posture could produce elevations of renal vein plasma renin in patients with a fixed renal artery lesion. This mechanism could be the basis for reports of increased renin concentrations in persons who have hypertension associated with renal artery pathology. Much more work must

be done however, before such a mechanism can be regarded as the pathogenesis of renal hypertension!

CONCLUSIONS

1. The sympathetic response following carotid occlusion in the mongrel dog stimulates the secretion of renin.
2. Control of the renal perfusion pressure during the sympathetic pressor response will result in a greater magnitude of renin secretion.
3. At least ten minutes are required to increase renal vein plasma renin levels above resting levels following carotid artery occlusion.
4. The maximal pressor response following carotid artery occlusion is not co-incident with the maximal output of renin and occurs three to ten minutes after occlusion of these vessels.
5. Sodium depletion in the mongrel dog results in elevated resting levels of renal vein renin concentrations. An apparent magnification of the "renin" release could not be verified statistically.

SUMMARY

Sodium depletion in the mongrel dog produces increased resting levels of renal vein "renin" activity.

The sympathetic response following occlusion of the carotid arteries causes a secretion of renin from canine kidneys which may be partially inhibited by the simultaneous increase in renal perfusion pressure. The mechanism of renin release following carotid occlusion is probably an indirect result of a decrease in glomerular filtration rate and sodium load to the distal convoluted tubule. There is no evidence that a direct neurological mechanism or one involving a renal baroreceptor was instrumental in the renin secretion following a sympathetic nervous system response in our dogs.

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A P P E N D I X

HEMODYNAMIC DATA (Control Group)

Dog No. E-1294 (Normal)

Minutes	0	+60	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	162 / 140 / 120	140 / 118 / 100	144 / 122 / 108	144 / 122 / 116
Pulse Pressure / $\bar{x}$ Perf. Press.	42 / 140	40 / 118	36 / 122	28 / 122
Heart Rate	126	138	138	132
Flow (mls/min)	212	225	225	218

Dog No. E-1471 (Na depleted)

Minutes	0	+60	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	132 / 104 / 92	116 / 88 / 80	96 / 76 / 70	84 / 68 / 62
Pulse Pressure / $\bar{x}$ Perf. Press.	40 / 104	36 / 86	20 / 74	22 / 68
Heart Rate	144	144	150	144
Flow (mls/min)	179	172	172	179

Dog No. E-1445 (Na depleted)

Minutes	0	+60	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	178 / 120 / 98	188 / 132 / 120	184 / 136 / 124	180 / 142 / 130
Pulse Pressure / $\bar{x}$ Perf. Press.	80 / 122	68 / 132	60 / 136	50 / 142
Heart Rate	156	156	162	162
Flow (mls/min)	136	136	142	149

HEMODYNAMIC DATA (Control Group)

Dog No. E-1677 (Na depleted)

Minutes	0	+60	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	144 / 108 / 95	136 / 96 / 80	140 / 100 / 86	136 / 98 / 86
Pulse Pressure / $\bar{x}$ Perf. Press.	49 / 108	56 / 96	54 / 100	50 / 100
Heart Rate	90	84	90	90
Flow (mls/min)	70	76	70	76

Dog No. E-1304 (Na depleted)

Systemic Pressure (systolic / diastolic / $\bar{x}$)	148 / 122 / 108	140 / 118 / 104	132 / 110 / 94	132 / 110 / 94
Pulse Pressure / $\bar{x}$ Perf. Press.	40 / 122	36 / 118	38 / 110	38 / 110
Heart Rate	126	120	120	126
Flow (mls/min)	122	122	127	122

Dog No. E-1377 (Na depleted)

Systemic Pressure (systolic / diastolic / $\bar{x}$)	138 / 100 / 80	130 / 102 / 80	136 / 102 / 88	138 / 104 / 94
Pulse Pressure / $\bar{x}$ Perf.Press.	58 / 100	50 / 102	48 / 102	46 / 104
Heart Rate	132	138	138	138
Flow (mls/min)	145	145	143	-

RENAL VEIN "RENIN" ACTIVITY

Minutes	0	30	90	120	180
Control Group					
*E-1294	+2.2, 1.5, 2.6	1.9, 1.1, .7	2.6, 1.9, 3.0	1.9, 1.1, 1.1	3.0, 3.7, 3.7
	= 2.1	1.2	2.5	1.4	3.5
E-1471	7.4, 6.7, 7.8	7.4, 9.3, 8.1	8.1, 7.0, 8.1	8.5, 7.8, 7.4	9.3,10.4,11.0
	7.3	8.3	7.7	7.9	10.2
E-1445	3.0, 2.6, 3.7	2.6, 1.5, 1.1	1.5, .7, 2.2	1.5, 2.6, 2.2	2.6, 1.9, 2.6
	3.1	1.4	1.4	2.1	2.4
E-1677	8.5, 7.4, 6.7	9.2, 7.5, 8.8	10.4,8.2 9.2	7.4, 5.9, 5.6	5.2, 5.9, 5.9
	7.5	8.5	9.2	6.3	5.7
E-1304	7.4, 8.1, 6.7	6.7, 5.5, 6.7	7.8, 6.7, 5.5	6.7, 5.4, 4.4	3.7, 4.4, 5.2
	7.4	6.3	6.7	5.5	4.4
E-1377	4.8, 5.5, 5.2	4.1, 3.7, 5.5	4.8, 5.5, 5.9	4.1, 4.1, 3.3	3.7, 4.1, 3.0
	5.1	4.4	5.4	3.8	3.6

* "Normal" dog.

\+ Three samples of "renin" activity expressed as ng./ml. of original plasma.

= Mean Renal Vein "renin" activity.

FLUIDS AND ELECTROLYTES (Control Group)

Minutes	0 to 60				60 to 120				120 to 180				Pooled Right Urine		Pooled Left Urine	
	Blood			Ur	Blood			Ur	Blood			Ur				
Dog #	Hgb*	Hct	Na†	Vol	Hgb	Hct	Na	Vol	Hgb	Hct	Na	Vol	Sp.Gr	Na	Sp.Gr	Na
E-1294	16	47	160	R20 L22	16	47	135	R9 L8	16	48	138	R8 L4				
E-1471	14	45	137	R12 L2	14	46	139	R9 L8	16	49	131	R4 L1	1.020	11	1.024	22
E-1445	17	52	124	R22 L12	17	53	139	R32 L9	17	54	144	R21 L1	1.010	22	1.026	6
E-1677	17	53	152	R2 L4	19	58	153	R1 L2	19	58	139	R1 L2		56		22
E-1304	15	46	137	R .8 L9	16	47	144	R L2	17	50	145	R L1				
E-1377	16	54	149	R7 L5	17	55	147	R12 L11	17	55	142	R5 L4	1.042	81	1.040	28

* g%

† mEqwt/litre.

GROUP I

HEMODYNAMIC DATA (Group I)

Dog No. E-1342

Minutes	0	+10	+30	+90	+100	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	160 / 122 / 108	276 / 218 / 188	200 / 148 / 122	142 / 114 / 100	264 / 218 / 200	218 / 176 / 160	120 / 98 / 88
Pulse Pressure / $\bar{x}$ Perf. Press	52 / 122	88 / 216	78 / 148	42 / 116	8 / 118	8 / 118	32 / 100
Heart rate	152	210	168	156	216	186	156
Flow (mls/min)	197	203	222	197	176	167	176

Dog No. E-1401

Minutes	0	+10	+30	+90	+100	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	120 / 86 / 70	178 / 142 / 128	190 / 148 / 124	138 / 104 / 92	228 / 190 / 152	190 / 160 / 140	144 / 120 / 108
Pulse Pressure / $\bar{x}$ Perf. Press.	50 / 88	48 / 140	66 / 108	46 / 108	20 / 110	20 / 110	36 / 126
Heart rate	126	132	126	126	156	138	126
Flow (mls/min)	-	-	-	-	-	-	-

Dog No. E-1000

Minutes	0	+10	+30	+90	+100	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	162 / 120 / 96	236 / 200 / 160	176 / 152 / 120	144 / 116 / 88	224 / 184 / 160	224 / 184 / 160	140 / 114 / 94
Pulse Pressure / $\bar{x}$ Perf.Press	66 / 118	76 / 200	56 / 152	56 / 112	12 / 115	12 / 115	46 / 112
Heart rate	138	132	132	116	126	126	114
Flow (mls/min	152	159	165	152	137	144	152

HEMODYNAMIC DATA (Group I)

Dog No. E-1004

Minutes	0	+10	+30	+90	+100	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	144 124 100	242 192 172	200 172 150	148 122 108	270 226 204	204 184 170	140 120 100
Pulse Pressure	44	70	50	40	8	8	40
$\bar{x}$ Perf. Press.	124	194	174	124	124	124	120
Heart Rate	120	126	126	126	144	144	126
Flow (mls/min)	105	88	88	105	70	59	82

Dog No. E-1394

Systemic Pressure (systolic / diastolic / $\bar{x}$)	110 90 70	142 106 86	134 102 84	106 80 68	158 126 118	144 122 115	112 84 72
Pulse Pressure	40	6	6	37	30	29	40
$\bar{x}$ Perf.Press.	92	90	90	88	130	118	80
Heart Rate	96	124	122	110	144	146	112
Flow (mls/min)	-	-	-	-	-	-	-

RENAL VEIN "RENIN" ACTIVITY

Minutes	0	30	90	120	180
Group I.					
E-1342	.7, .7, .4 .6	3.3, 4.1, 3.0 3.4	.7, 1.5, 1.1 1.1	3.7, 4.1, 4.1 3.9	.4, .7, .7 .6
E-1401	2.6, 1.9, 3.0 2.5	4.4, 3.7, 4.4 4.2	1.1, .7, .7 .8	5.5, 6.3, 5.2 5.6	2.2, 3.0, 2.6 2.6
E-1000	.4, .4, .7 .5	1.9, 1.1, 1.1 1.4	1.1, .7, 1.5 1.1	6.3, 7.0, 5.9 6.4	3.7, 4.1, 3.7 3.8
E-1004	.7, .7, 1.1 .8	3.0, 3.3, 2.6 3.0	1.9, 1.1, 1.5 1.5	8.5, 9.3, 8.1 8.6	3.3, 3.3, 3.7 3.4
E-1394	1.9, 1.9, 1.1 1.6	8.5, 8.9, 8.1 8.5	1.5, 1.9, 1.9 1.8	3.7, 3.7, 3.0 3.5	.7, 1.1, 1.1 .9
Group II.					
E-1562	2.6, 1.9, 1.9 2.1	1.1, 1.1, .7 .9	1.9, 2.6, 1.5 2.0	9.3, 8.9, 9.3 9.2	2.6, 1.9, 1.9 2.1
E-1615	2.6, 3.7, 3.7 3.3	5.5, 4.8, 5.9 5.4	2.2, 1.9, 1.9 2.0	6.7, 7.4, 7.4 7.2	5.5, 4.4, 4.4 4.8
E-1597	3.7, 4.4, 3.7 3.9	1.9, 2.6, 2.2 2.2	1.1, 1.5, 1.5 1.4	9.3, 9.3, 8.1 8.9	3.0, 2.6, 2.6 2.7
E-1622	1.5, 1.5, 1.9 1.6	11.8,11.0,11.0 11.3	1.9, 2.6, 2.6 2.4	1.9, 1.9, 1.1 1.6	2.6, 3.0, 2.6 2.7
E-1602	3.7, 3.7, 4.1 3.8	8.9, 9.3, 8.5 8.9	2.6, 3.0, 3.0 2.9	11.8,11.0,11.0 11.3	3.0, 3.7, 3.7 3.5

FLUIDS AND ELECTROLYTES (Group I)

Minutes	0 to 60						60 to 120						120 to 180					
	Blood			Urine			Blood			Urine			Blood			Urine		
Dog #	Hgb	Hct	Na	Vol	Na	Sp.Gr	Hgb	Hct	Na	Vol	Na	Sp.Gr	Hgb	Hct	Na	Vol	Na	Sp.Gr
E-1394	15	45	161	R2 L8	62 13	-	14	45	137	R .5 L .9	46 30	-	15	44	144	R .1 L14	23	-
E-1342	18	55	156	R5 L3	199 114	-	17	52	157	R10 L5	88 19	-	17	51	147	R5 L4	32 18	-
E-1401	14	43	142	R6 L7	124 119	-	14	44	147	R5 L6	122 105	-	16		136	R5 L5	151 109	-
E-1004	18	54	142	R6 L7	56 115	1.024 1.024	18	53	142	R5 L6	30 48	1.024 1.024	18	53	147	R5 L .5	27 56	1.024 -
E-1000	15	47	157	R5 L7	135 122	1.020 1.020	15	45	147	R9 L10	62 70	1.028 1.028	13	41	167	R12 L25	69 57	1.032 1.032

GROUP II

HEMODYNAMIC DATA (Group II)

Dog No. E-1562

Minutes	0	+10	+30	+90	+100	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	116 / 92 / 80	180 / 154 / 146	166 / 142 / 132	112 / 98 / 90	182 / 158 / 150	182 / 158 / 150	120 / 110 / 100
Pulse Pressure / $\bar{x}$ Perf. Press.	36 / 94	34 / 154	34 / 146	18 / 100	8 / 100	8 / 100	20 / 110
Heart Rate	144	180	168	138	162	162	138
Flow (mls/min)	182	174	174	199	149	141	182

Dog No. E-1615

Minutes	0	+10	+30	+90	+100	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	110 / 86 / 76	122 / 98 / 88	116 / 92 / 84	112 / 90 / 80	144 / 128 / 122	122 / 112 / 98	112 / 100 / 90
Pulse Pressure / $\bar{x}$ Perf. Press.	34 / 86	34 / 96	32 / 92	32 / 92	12 / 94	12 / 90	22 / 100
Heart Rate	120	132	138	132	156	156	114
Flow (mls/min)	-	-	-	-	-	-	-

Dog No. E-1597

Minutes	0	+10	+30	+90	+100	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	140 / 118 / 104	210 / 184 / 166	160 / 132 / 120	132 / 110 / 94	186 / 150 / 132	184 / 156 / 140	138 / 108 / 100
Pulse Pressure / $\bar{x}$ Perf. Press.	36 / 120	44 / 184	40 / 134	38 / 114	18 / 118	18 / 118	38 / 110
Heart Rate	174	228	204	174	192	192	168
Flow (mls/min)	-	-	-	-	-	-	-

HEMODYNAMIC DATA (Group II)

Dog No. E-1622

Minutes	0	+10	+30	+90	+100	+120	+180
Systemic Pressure ($\frac{\text{systolic}}{\text{diastolic}}$ $\bar{x}$)	144 / 118 / 108	242 / 198 / 188	160 / 140 / 130	136 / 116 / 108	190 / 168 / 156	160 / 140 / 130	112 / 90 / 80
Pulse Pressure / $\bar{x}$ Perf. Press.	36 / 116	6 / 112	6 / 112	28 / 118	34 / 170	30 / 144	32 / 90
Heart Rate	158	210	192	168	192	180	144
Flow (mls/min)	147	139	147	139	131	139	147

Dog No. E-1602

Minutes	0	+10	+30	+90	+100	+120	+180
Systemic Pressure ($\frac{\text{systolic}}{\text{diastolic}}$ $\bar{x}$)	94 / 76 / 68	136 / 116 / 104	112 / 98 / 90	90 / 74 / 60	116 / 96 / 82	116 / 94 / 80	92 / 78 / 62
Pulse Pressure / $\bar{x}$ Perf. Press.	26 / 74	8 / 88	8 / 84	30 / 72	34 / 94	36 / 94	30 / 80
Heart Rate	134	156	144	120	138	138	126
Flow (mls/min)	-	-	-	-	-	-	-

FLUIDS AND ELECTROLYTES (Group II)

Minutes	0 to 60				60 to 120				120 to 180				Pooled Right Urine		Pooled Left Urine	
	Blood			Ur	Blood			Ur	Blood			Ur				
Dog #	Hgb	Hct	Na	Vol	Hgb	Hct	Na	Vol	Hgb	Hct	Na	Vol	Sp.Gr	Na	Sp.Gr	Na
E-1562	19	60	143	R L3	19	60	148	R L3	19	61	143	R L5			1.040	10.5
E-1615	16	47	148	R .6 L6	15	45	157	R L5	16	47	145	R L3		57	1.046	16
E-1602	16	52	145	R2 L .2	17	54	145	R2 L .5	16	52	158	R4 L6	1.030	90	1.04	52
E-1597	17	54	167	R20 L6	17	54	169	R4 L5	16	53	159	R L1	1.018	30	1.020	25
E-1622	18	55	140	R5 L .3	19	59	168	R3 L2	18	57	167	R5 L2	1.020	41	1.030	46

GROUP III

HEMODYNAMIC DATA (Group III)

Dog No. E-1531

Minutes	0	+10	+30	+90	+100	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	132 / 112 / 100	260 / 190 / 150 (110)	200 / 166 / 140	140 / 102 / 90	220 / 176 / 150	190 / 166 / 140	120 / 96 / 80
Pulse Pressure	32	100	60	50	8	8	40
$\bar{x}$ Perf. Press.	116	190	166	102	108	108	96
Heart Rate	144	192	192	162	204	180	156
Flow (mls/min)	-	-	-	194	164	164	178

Dog No. E-1299

Systemic Pressure (systolic / diastolic / $\bar{x}$)	140 / 108 / 88	220 / 187 / 150	210 / 170 / 140	126 / 96 / 78	200 / 174 / 144	190 / 164 / 138	134 / 102 / 80
Pulse Pressure	52	70	70	48	10	10	54
$\bar{x}$ Perf. Press.	108	187	170	96	100	100	100
Heart Rate	114	150	138	124	144	144	120
Flow (mls/min)	104	126	126	98	87	98	109

Dog No. E-1373

Systemic Pressure (systolic / diastolic / $\bar{x}$)	146 / 118 / 102	184 / 150 / 136	180 / 148 / 136	150 / 120 / 104	240 / 194 / 176	216 / 190 / 158	144 / 118 / 106
Pulse Pressure	44	48	44	46	12	12	48
$\bar{x}$ Perf. Press.	118	146	146	118	118	118	118
Heart Rate	126	144	144	126	144	150	132
Flow (mls/min)	194	203	203	203	182	182	223

HEMODYNAMIC DATA (Group III)

Dog No. E-1486

Minutes	0	+10	+30	+90	+100	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	146 / 112 / 100	280 / 210 / 180	160 / 138 / 125	136 / 116 / 108	190 / 166 / 150	186 / 164 / 152	102 / 86 / 74
Pulse Pressure / $\bar{x}$ Perf. Press.	46 / 112	100 / 210	35 / 136	28 / 114	8 / 115	8 / 115	28 / 88
Heart Rate	114	180	142	120	132	132	120
Flow (mls/min)	238	258	253	211	181	188	197

Dog No. E-1489

Minutes	0	+10	+30	+90	+100	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	118 / 88 / 78	260 / 195 / 180	200 / 170 / 140	126 / 92 / 78	184 / 140 / 126	196 / 150 / 120	116 / 90 / 72
Pulse Pressure / $\bar{x}$ Perf. Press.	40 / 88	10 / 90	10 / 88	48 / 94	62 / 144	76 / 152	44 / 90
Heart Rate	126	150	138	120	132	126	120
Flow (mls/min)	145	123	131	138	159	153	138

Dog No. E-1572

Minutes	0	+10	+30	+90	+100	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	164 / 140 / 124	288 / 240 / 218	240 / 210 / 190	158 / 136 / 128	220 / 178 / 160	220 / 178 / 160	160 / 122 / 108
Pulse Pressure / $\bar{x}$ Perf. Press.	40 / 142	6 / 142	6 / 142	30 / 138	60 / 180	60 / 180	52 / 124
Heart Rate	150	168	162	132	138	138	144
Flow (mls/min)	140	131	140	140	164	164	140

HEMODYNAMIC DATA (Group III)

Dog No. E-1361

Minutes	0	+10	+30	+90	+100	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	146 / 112 / 92	312 / 280 / 200	220 / 180 / 150	140 / 112 / 86	240 / 212 / 180	220 / 180 / 150	138 / 116 / 116
Pulse Pressure	54	10	10	54	60	70	44
$\bar{x}$ Perf. Press.	112	112	112	112	212	180	116
Heart Rate	144	186	180	156	186	174	150
Flow (mls/min)	166	123	108	138	138	130	143

Dog No. E-1368

Minutes	0	+10	+30	+90	+100	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	144 / 108 / 84	210 / 170 / 140	170 / 130 / 100	122 / 92 / 74	166 / 126 / 100	160 / 118 / 96	120 / 90 / 72
Pulse Pressure	64	12	12	58	66	84	48
$\bar{x}$ Perf. Press.	108	110	104	92	122	118	92
Heart Rate	138	180	150	132	150	138	120
Flow (mls/min)	128	116	128	128	134	128	128

RENAL VEIN "RENIN" ACTIVITY

Minutes	0	30	90	120	180
Group III.					
E-1531	1.5, 1.9, 1.9 1.8	.7, .7, 1.1 .8	1.1, 1.1, .7 1.0	4.4, 5.2, 3.7 4.4	.7, .7, .4 .6
E-1299	5.5, 5.9, 4.4 5.3	14.8,15.5,15.9 15.4	5.5, 4.8, 5.5 5.3	16.3,14.8,16.3 15.8	5.5, 4.4, 5.2 5.0
E-1373	5.5, 5.9, 5.9 5.8	11.0,11.0,11.8 11.3	5.5, 4.4, 4.4 4.8	9.3, 8.1, 9.3 8.9	2.6, 3.0, 3.0 2.9
E-1486	1.9, 1.5, 1.9 1.7	9.3,10.4, 9.6 9.8	1.9, 1.5, 1.5 1.6	7.4, 8.1, 7.4 7.6	2.2, 1.5, 2.2 2.0
E-1489	.7, 1.9, 1.9 1.5	2.2, 1.9, 2.6 2.2	3.7, 3.0, 3.7 3.5	.4, .4, .7 .5	1.5, 1.1, 1.1 1.2
E-1572	2.2, 1.9, 2.6 2.2	8.5, 9.3, 8.5 8.8	3.7, 3.0, 3.7 3.5	4.4, 4.8, 3.7 4.3	1.9, 1.9, 2.2 2.0
E-1361	2.2, 3.0, 2.2 2.5	18.5,19.3,18.5 18.8	1.5, 2.2, 1.9 1.8	10, 10.7, 10 10.2	1.9, 2.6, 2.6 2.4
E-1368	4.4, 3.7, 3.7 3.9	13.3,13.0,11.8 12.7	5.5, 4.4, 5.5 5.1	18.5,17.8,19.3 18.5	8.9, 8.1, 8.1 8.4

FLUIDS AND ELECTROLYTES (Group III)

Minutes	0 to 60				60 to 120				120 to 180				Pooled Right Urine		Pooled Left Urine	
	Blood			Ur	Blood			Ur	Blood			Ur				
Dog #	Hgb	Hct	Na	Vol	Hgb	Hct	Na	Vol	Hgb	Hct	Na	Vol	Sp.Gr	Na	Sp.Gr	Na
E-1351	14	47	135	R38 L16	14	47	133	R7 L5	14	47	143	R4 L5	1.010	101	1.030	37
E-1468	17	54	134	R5 L6	17	54	136	R .5 L3	18	58	142	R .5 L4	1.032	26	1.028	24
E-1373	18	56		R L5	18	54	150	R L7	18	54	136	R L13			1.020	23
E-1299	15	45	146	R28 L22	15	45	143	R36 L20	14	42	145	R30 L11	1.020		1.020	
E-1486	18	53	120	R1 L5	18	52	126	R7 L17	19	56	135	R2 L2	1.030	21	1.032	29
E-1572	18	56	139	R .4 L2	18	58	142	R2 L .4	17	53	155	R2 L1	1.040	56	1.050	30
E-1361	17	52	144	R7 L7	17	52	143	R4 L10	17	50	139	R3 L13	1.020	30	1.022	19
E-1368	17	50	140	R2 L4	17	50	139	R2 L2	17	50	114	R4 L9	1.030	21	1.040	13

GROUP IV

HEMODYNAMIC DATA (Group IV)

Dog No. E-1678

Minutes	0	+25	+30	+90	+115	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	138 / 116 / 96	210 / 190 / 160	190 / 160 / 140	142 / 118 / 106	240 / 210 / 190	210 / 186 / 170	140 / 120 / 104
Pulse Pressure / $\bar{x}$ Perf. Press.	42 / 116	50 / 190	50 / 160	36 / 120	8 / 120	8 / 120	36 / 120
Heart Rate	156	168	174	160	198	168	156
Flow (mls/min)	212	221	243	212	174	174	205

Dog No. E-1706

Minutes	0	+25	+30	+90	+115	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	130 / 100 / 88	160 / 138 / 120	160 / 134 / 120	150 / 110 / 90	210 / 170 / 154	200 / 164 / 140	148 / 118 / 100
Pulse Pressure / $\bar{x}$ Perf. Press.	42 / 102	40 / 140	40 / 136	60 / 110	12 / 116	12 / 120	48 / 120
Heart Rate	150	150	156	144	168	156	156
Flow (mls/min)	159	145	136	165	120	145	159

Dog No. E-1704

Minutes	0	+25	+30	+90	+115	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	112 / 88 / 80	148 / 120 / 112	148 / 118 / 88	114 / 86 / 80	128 / 110 / 100	120 / 100 / 90	108 / 82 / 70
Pulse Pressure / $\bar{x}$ Perf. Press.	32 / 88	36 / 124	60 / 120	34 / 86	12 / 90	12 / 90	38 / 82
Heart Rate	132	138	138	144	150	138	144
Flow (mls/min)	-	-	-	-	-	-	-

HEMODYNAMIC DATA (Group IV)

Dog No. E-1664

Minutes	0	+25	+30	+90	+115	+120	+180
Systemic Pressure (systolic / diastolic / $\bar{x}$)	140 116 106	188 156 150	190 156 148	120 102 92	160 144 128	116 95 88	128 102 100
Pulse Pressure	34	12	12	28	32	28	28
$\bar{x}$ Perf. Press.	120	120	120	106	142	142	104
Heart Rate	162	204	186	162	186	156	160
Flow (mls/min)	143	128	136	97	81	88	-

Dog No. E-1670

Systemic Pressure (systolic / diastolic / $\bar{x}$)	128 90 80	200 168 146	190 160 144	120 88 80	160 130 120	162 134 122	120 90 80
Pulse Pressure	48	8	8	40	40	40	40
$\bar{x}$ Perf. Press.	90	90	90	88	130	134	90
Heart Rate	132	144	144	126	144	138	126
Flow (mls/min)	235	181	181	-	-	-	-

RENAL VEIN "RENIN" ACTIVITY

Minutes	0	30	90	120	180
Group IV.					
E-1678	1.9, 2.6, 3.0 2.5	3.7, 3.7, 4.4 3.9	3.0, 1.9, 3.0 2.6	3.7, 4.4, 3.7 3.9	5.2, 4.4, 5.5 5.0
E-1706	3.3, 4.8, 5.2 4.4	1.5, 2.2, 3.0 2.2	2.6, 2.2, 2.2 2.3	3.0, 3.7, 4.1 3.6	4.4, 3.7, 4.4 4.2
E-1704	9.2, 7.4, 8.2 8.3	8.2, 9.2, 9.2 8.9	6.4, 5.9, 5.6 5.9	5.6, 5.2, 5.6 5.5	4.4, 5.2, 4.4 4.7
E-1664	2.6, 3.0, 1.9 2.5	3.0, 3.0, 2.2 2.7	1.9, 1.9, 1.5 1.8	.4, 1.1, .7 .7	1.9, 2.2, 2.2 2.1
E-1670	3.0, 3.0, 3.3 3.1	1.1, 1.5, 2.2 1.6	1.9, 1.9, 1.1 1.6	.7, 1.1, .7 .8	1.9, 2.2, 2.2 2.1

FLUIDS AND ELECTROLYTES (Group IV)

Minutes	0 to 60				60 to 120				120 to 180				Pooled Right Urine		Pooled Left Urine	
	Blood			Ur	Blood			Ur	Blood			Ur				
Dog #	Hgb	Hct	Na	Vol	Hgb	Hct	Na	Vol	Hgb	Hct	Na	Vol	Sp.Gr	Na	Sp.Gr	Na
E-1664	19	58	168	R .2 L3	17	54	178	R1 L2	19	58	163	R .8 L2	1.030	37	1.040	24
E-1670	15	47	159	R .5 L .5	14	46	157	R .2 L .1	15	46	146	R2 L0		107		98
E-1678	16	48	159	R3 L2	16	49	153	R2 L1	17	52	154	R5 L2	1.030	68		53
E-1706	17	51	154	R3 L .8	17	52	173	R4 L6	18	54	146	R3 L4	1.020	149	1.030	77
E-1704	17	52	161	R1 L3	18	55	162	R .1 L4	18	55	146	R1 L .5		34		39

SODIUM EXCRETION IN TWENTY-THREE SODIUM DEPLETED DOGS

		Na mEqwts/24 hours
A.	Controls	
	E-1304	0.14
	E-1377	4.20
	E-1445	30.00
	E-1677	1.4
	E-1471	8.8
B.	Group II	
	E-1562	2.7
	E-1615	0.7
	E-1597	1.1
	E-1602	4.7
	E-1622	1.7
C.	Group III	
	E-1531	1.0
	E-1486	0.6
	E-1299	0.5
	E-1373	2.8
	E-1368	0.6
	E-1361	0.5
	E-1572	5.7
	E-1489	2.3

D. Group IV

E-1678	1.4
E-1670	1.6
E-1664	2.3
E-1704	3.5
E-1706	3.0